International Journal of Hygiene and Environmental Health 223 (2020) 93–105

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

Changes in urinary excretion of phthalates, phthalate substitutes, bisphenols and other polychlorinated and phenolic substances in young Danish men; 2009–2017

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ARTICLE INFO

Keywords:
Endocrine disruptor
Human biomonitoring
Phthalate
DINCH
Di-2-ethylhexyl terephthalate (DEHTP)
Phenol

ABSTRACT

During the past two decades human exposure to bisphenol A (BPA) and phthalates such as di-iso-butyl phthalate (DiBP), di-n-butyl phthalate (DnBP), butylbenzyl phthalate (BBzP) and di-(2-ethyl-hexyl) phthalate (DEHP) has received substantial interest due to widespread population exposures and potential endocrine disrupting effects. Therefore, these chemicals have gradually been restricted and phased out through legislation. However, humans are still exposed to a wide range of other less studied phthalates, phthalate substitutes and BPA analogues as well as other polychlorinated and phenolic substances.

In this study, we investigated human exposure to these chemicals over the past decade. Three hundred urine samples collected in 2009, 2013 and 2017 (100 samples each year) from young Danish men of the general population, participating in a large on-going cross-sectional study, were selected for the present time trend study. The urinary concentration of metabolites of 15 phthalates, di-2-ethylhexyl terephthalate (DEHTP) and di-iso-nonyl-cyclohexane-1,2-dicarboxylate (DINCH), seven bisphenols including BPA, bisphenol S (BPS) and bisphenol F (BPF), as well as triclosan, triclocarban, benzophenone-3, three chlorophenols and two phenylphenols were analyzed by two new sensitive LC-MS/MS methods developed and validated for the present study.

A significant decrease in urinary concentrations over time was observed for the majority of the chemicals. Median concentrations of BPA and the metabolites of DiBP, DnBP, BBzP and DEHP were more than halved from 2009 to 2017. Similar decreases were observed for triclosan and the chloro- and phenylphenols. In contrast, metabolites of the two phthalate substitutes DEHTP and DINCH increased more than 20 and 2 times, respectively. The potential BPA substitutes; BPS and BPF also increased, but only slightly. Despite these new exposure patterns, the exposure to the old well-known chemicals, such as DiBP, DnBP, BBzP, DEHP and BPA was still higher in 2017 compared to the exposure level of the new substitutes such as DEHTP, DINCH, BPS and BPF.

A significant decrease in internal exposure to most of the common phthalates and BPA over the past decade was observed, reflecting market changes and regulatory measures implemented in EU. Despite increasing exposures to some of the known phthalate substitutes and BPA analogues, the total amount of each measured chemical group (original and substitute analytes combined) was lower in the more recently collected samples. This indicates only partial direct substitution or substitution by chemicals not covered in this approach, or a general decline in the exposure to these chemical/product groups over the last decade.

1. Introduction

Humans in modern societies are exposed to thousands of synthetic industrial chemicals throughout life, either voluntarily by direct product use or involuntarily by environmental contamination. Among these chemicals the large group of phthalate diesters (phthalates) and bisphenol A (BPA) and other polychlorinated and phenolic substances such as triclosan, triclocarban, benzophenone-3, 2,4-dichlorophenol,

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https://doi.org/10.1016/j.ijheh.2019.10.002
Received 20 June 2019; Received in revised form 6 October 2019; Accepted 6 October 2019

2,5-dichlorophenol, 2,4,5-trichlorophenol, 2-phenylphenol and 4-phenylphenol are of concern because of their endocrine disrupting potentials. Human biomonitoring (HBM) studies have unequivocally shown that human populations worldwide are exposed to most of these man-made chemicals (CDC, 2019; Frederiksen et al., 2014; Gyllenhammar et al., 2017; Haug et al., 2018; Koch et al., 2017). Phthalates and many polychlorinated and phenolic substances are rapidly metabolized and excreted through urine or faeces over the course of a few days and are classified as non-persistent or partly non-persistent because of their short biological half-lives. However, due to continuously exposure, through ingestion, inhalation or by dermal contact, many of these chemicals are also called pseudo-persistent (Koch and Calafat, 2009).

Phthalates are present in numerous consumer products and widely used as plasticizers and additives in industrial products (Serrano et al., 2014). BPA is used in polycarbonate plastics and epoxy resins as well as an additive in other formulations and thus present in numerous consumer products including clothes, linings of canned food packaging and products produced of recycling materials. BPA is among the highest production volume chemicals worldwide (Xue et al., 2017). Triclosan and triclocarban are primarily used as antimicrobial and bacterial agents in consumer and personal care products, like toothpaste and antimicrobial soaps (Dann and Hontela, 2011; Environment and Climate Change Canada, 2016). Benzophenone-3 is used as UV filter in sunscreens and other personal care products as well as in many other consumer products, such as packaging materials, clothes, furniture textiles and paint (The Danish Environmental Protection Agency, 2015; Xue et al., 2017). Chlorophenols such as 2,4-dichlorophenol, 2,5-dichlorophenol and 2,4,5-trichlorophenol are pesticides and have also been used as intermediates in the industrial production of especially herbicides (ASTDR, 1999). 2,5-dichlorophenol is the major urinary metabolite of p-dichlorobenzene, which is used for disinfection and as a pesticide (Yoshida et al., 2002) and 2,4-dichlorophenol can be used in synthesis of triclosan and is also a photo-degradation product of triclosan (Latch et al., 2005). 2-phenylphenol and 4-phenylphenol are used as post-harvest fungicides in the fruit growing industry and disinfectant in industrial and household products (Agency, 2006).

Several animal studies as well as human epidemiologic studies have shown or indicated antiandrogenic and/or estrogenic effects of some commonly used phthalates such as di-iso-butyl phthalate (DiBP), di-n-butyl phthalate (DnBP), butylbenzyl phthalate (BBzP), di-(2-ethylhexyl) phthalate (DEHP) and di-iso-nonyl phthalate (DiNP), as well as BPA, triclosan and benzophenone-3 (Dann and Hontela, 2011; Environment and Climate Change Canada, 2016; Howdeshell et al., 2017; Kinnberg et al., 2015; Krause et al., 2012; Lassen et al., 2014; Prins et al., 2018; Radke et al., 2018; Rehfeld et al., 2018; van den Driesche et al., 2017; Vandenberg et al., 2019).

Due to concerns regarding the endocrine disrupting abilities of BPA and the phthalates DiBP, DnBP, BBzP and DEHP, their use in toys and childcare products, personal care products and food contact materials has gradually been restricted during the past decades by national and international legislation. All five substances are included on The European Commission's REACH (Regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals) candidate list of Substance of very high concern (SVHC) based on their reprotoxicity and endocrine disrupting properties (ECHA candidate list, 2019). In July 2018, The European Commission decided further restrictions in the use of the four phthalates in consumer products on the EU market and from July, 2020 these substances will be restricted to a concentration equal to or below 0.1% by weight individually or in any combination in any plasticized material in articles used by consumers or in indoor areas (EU_Commission_Regulation_phthlates, 2018). Additionally, di-iso-nonyl phthalate (DINP), di-iso-decyl phthalate (DIDP) and di-n-octyl phthalate (DnOP) are restricted under the REACH legislation for similar properties, while the pentyl phthalates (DnPeP, DiPeP, Dn/iPeP) are included on the REACH candidate list as SVHC substances (ECHA candidate list, 2019).

In parallel to the phase out of some phthalates, new substances such as di-2-ethylhexyl terephthalate (DEHTP) and di-iso-nonyl-cyclohexane-1,2-dicarboxylate (DINCH) have been introduced as substitutes for DEHP and other high molecular phthalates with endocrine disrupting effects. Both DINCH and DEHTP have structural analogies to the phthalates and are metabolized and excreted in an analogous way (Koch et al., 2013; Lessmann et al., 2016a). The number of HBM studies including DINCH and DEHTP data are still limited but widespread exposure to both of these substitutes have been shown (Correia-Sa et al., 2017; Fromme et al., 2016; Kasper-Sonnenberg et al., 2019; Larsson et al., 2017; Lessmann et al., 2017, 2019; Silva et al., 2019). So far neither DINCH nor DEHTP have shown reprotoxic or endocrine disrupting properties in vitro or in vivo and are therefore considered possible safe alternatives to e.g. DEHP and DiNP (EFSA_DEHTP, 2008; EFSA_DINCP, 2006). Several analogues to BPA, e.g. bisphenol S (BPS) and bisphenol F (BPF) have been introduced on the market (Chen et al., 2016). However, little is known about the potential adverse effects of these increasingly used bisphenols but some of them seem to affect hormone systems similar to BPA (Eladak et al., 2015; Kojima et al., 2019).

Recent changes in legislation and regulation and maybe also voluntary phase out and substitution by the producers have likely changed the exposure patterns to phthalates and bisphenols. Furthermore, exposure data for the less-studied phthalates, the phthalate substitutes DEHTP and DINCH, and the BPA analogues are still scarce. Therefore, HBM studies are highly needed to follow the changes in populations exposure to these chemicals. Here we present changes over time in the urinary excretion of 32 chemicals measured in samples collected in 2009-17 from young Danish men. To achieve this, we expanded our existing analytical methodologies to two new fully validated LC-MS/MS methods including 1) metabolites of 15 phthalates, DEHTP and DINCH and 2) BPA and six bisphenol analogues together with polychlorinated and phenolic substances.

2. Materials and methods

2.1. Subjects and sample collection

In a large ongoing study on reproductive health of young men from the general Danish population, approximately 300 men with a mean age of 20 years (range 18–30 years) have been enrolled every year during the past 25 years. The men were recruited for participation in the main study when they were attending a mandatory screening for military enrollment eligibility in the Greater Copenhagen area, the capital region in Denmark. This means that all healthy young men in Denmark, with no exceptions and regardless of their background, attend this screening. In short, following informed consent, the men who accepted participation provided among other samples a spot urine sample, answered a questionnaire and underwent a physical examination, all in one day. Details of this study have previously been published (Jorgensen et al., 2012). For the present study, the first 100 urine samples from each of the study years 2009, 2013 and 2017 were selected. Thereby all samples were collected from February to April except eight samples from 2013, which were collected at the beginning of May. Immediately after collection aliquots of urine samples were decanted into 20 mL scintillation veils and stored at −20 °C until chemical analysis. Characteristics of the men are shown in Table 1. There were no significant differences between the characteristics of the selected men and the not selected men in the study group (data not shown).

All participants were informed about the study orally and in writing, and informed consent was signed individually prior to participation. This study was conducted in accordance with the principles of the Helsinki II declaration and was approved by the local Ethical Committees of the Capital Region (RegionH): journal no. H-KF-289428 and the Danish Data Protection Agency: no. RH-2015-246.

Table 1
Characteristics of cohort subjects, mean (range), participating in the study of young men; 2009, 2013 and 2017.

	All (n = 300)		2009 (n = 100)		2013 (n = 100)		2017 (n = 100)	
Age (year)	20.0	(17.9–30.3)	19.8	(18.1–25.1)	19.8	(17.8–26.7)	20.6	(19.1–30.3)
Height (cm)	183	(164–206)	182	(164–197)	184	(169–206)	183	(168–198)
Weight (kg)	75.9	(44.8–114)	75.6	(55.0–113)	76.1	(53.1–107)	75.9	(44.8–114)
BMI (kg/m^2)	22.7	(15.6–35.8)	22.9	(18.4–32.8)	22.6	(16.2–29.1)	22.6	(15.6–35.8)

2.2. Chemical analysis

Urine samples (n = 300) were analyzed by two recently developed and validated isotope dilution TurboFlow-LC-MS/MS methods: the phthalate method included metabolites of phthalates, DEHTP and DINCH, and the phenol method included bisphenols, polychlorinated and phenolic substances. The method for preparation of urine samples, standard solutions and quality controls, as well as the instrumental analysis and general method validation were based on our previous methods for measuring, respectively, phthalate metabolites and phenols in urine (Frederiksen et al., 2010, 2013a). All chemicals and metabolites measured in the present study are listed with name and abbreviation in Tables 2.1 and 2.2; from now on only abbreviations are used. The phthalate method was expanded to include in total 38 metabolites from 15 different phthalate diesters and the two phthalate substitutes DEHTP and DINCH (Table 2.1). The original method (Frederiksen et al., 2010) was modified by using online-TurboFlow-LC-MS/MS technology equipped with a probe for heated electrospray ionization (HESI) running in negative mode. To reach baseline separation of all metabolites the instrument run time was extended from 24 to 35 min. The phenol method (Frederiksen et al., 2013a) was expanded to include seven bisphenols together with eight chlorinated and phenolic substances (Table 2.2). The preceding enzymatic de-conjugation of the phthalates was done by arylsulfatase free β-glucuronidase (*Escherichia coli* K12), while the phenols were deconjugated by a mixture of β-glucuronidase (*Escherichia coli* K12) and sulfatase from Aerobacter Aerogenes. Detailed descriptions for both methods including standards and other materials and equipment, sample preparation, method validation, limits of detections (LOD), linear range, matrix effects, intra-day and inter-day accuracy and precision are included in Supplemental

Table 2.1
Phthalate diesters, phthalate substitutes and their metabolites.

Phthalate diester	Abbreviation	Human urine metabolite	Abbreviation	LOD (ng/mL)
Phthalates				
Di-methyl phthalate	DMP	Mono-methyl phthalate	MMP	0.27
Di-ethyl phthalate	DEP	Mono-ethyl phthalate	MEP	0.43
Di-iso-propyl phthalate	DiPrP	Mono-iso-propyl phthalate	MiPrP	0.07
	DnPrP	Mono-n-propyl phthalate	MnPrP	0.11
Di-iso-butyl phthalate	DiBP	Mono-iso-butyl phthalate	MiBP	0.16
		Mono-(2-hydroxy-iso-butyl) phthalate	2OH-MiBP	0.25
Di-n-butyl phthalate	DnBP	Mono-n-butyl phthalate	MnBP	0.17
		Mono-(3-hydroxybutyl) phthalate	3OH-MnBP	0.25
Butylbenzyl phthalate	BBzP	Mono-benzyl phthalate	MBzP	0.03
Di-n-pentyl phthalate	DnPeP	Mono-n-pentyl phthalate	MnPeP	0.04
Di-(2-ethyl-hexyl) phthalate	DEHP	Mono-(2-ethyl-hexyl) phthalate	MEHP	0.07
		Mono-(2-ethyl-5-hydroxyhexyl) phthalate	5OH-MEHP	0.05
		Mono- (2-ethyl-5-oxohexyl) phthalate	5oxo-MEHP	0.06
		Mono-(2-ethyl-5-carboxypentyl) phthalate	5cx-MEPP	0.07
		Mono-(2-carboxymethyl-hexyl) phthalate	2cx-MMHP	0.12
Di-n-hexyl phthalate	DHxP	Mono-n-hexyl phthalate	MnHxP	0.04
		Mono-(5-hydroxyhexyl) phthalate	OH-MHxP	0.02
		Mono-(5-carboxypentyl) phthalate	cx-MPeP	0.02
Di-cyclohexyl phthalate	DCHP	Mono-cyclohexyl phthalate	MCHP	0.23
Di-n-heptyl phthalate	DHpP	Mono-n-heptyl phthalate	MnHpP	0.03
		Mono-(6-hydroxyheptyl) phthalate	OH-MHpP	0.01
		Mono-(6-carboxyhexyl) phthalate	cx-MHxP	0.03
Di-octyl phthalate	DnOP	Mono-octyl phthalate	MnOP	0.03
		Mono-3-carboxypropyl phthalate	MCPP[a]	0.03
Di-iso-nonyl phthalate	DiNP	Mono-iso-nonyl phthalate	MiNP	0.14
		Mono-hydroxy-iso-nonyl phthalate	OH-MiNP	0.03
		Mono-oxo-iso-nonyl phthalate	oxo-MiNP	0.02
		Mono-carboxy-iso-octyl phthalate	cx-MiOP	0.04
Di-iso-decylphthalate	DiDP	Mono-iso-decyl phthalate	MiDP	0.16
		Mono-(9-hydroxydecyl) phthalate	OH-MiDP	0.02
		Mono-(9-oxodecyl) phthalate	oxo-MiDP	0.02
		Mono-(9-carboxynonyl) phthalate	cx-MiNP	0.02
Phthalate substitutes				
Di-2-ethylhexyl terephthalate	DEHTP	Mono-(2-ethyl-5-hydroxy-hexyl) terephthalate	5OH-MEHTP	0.03
		Mono-(2-ethyl-5-oxo-hexyl) terephthalate	5oxo-MEHTP	0.02
		Mono-(2-ethyl-5-caboxyl-pentyl) terephthalate	5cx-MEPTP	0.04
		Mono-(2-caboxyl-methyl-hexyl) terephthalate	2cx-MMHTP	0.03
Di-iso-nonyl-cyclohexane-1,2-dicarboxylate	DINCH	Cyclohexane-1,2-dicarboxylate-mono-(hydroxyl-iso-nonyl) ester	OH-MINCH	0.02
		Cyclohexane-1,2-dicarboxylate-mono-(carboxy-iso-octyl) ester	cx-MINCH	0.02

LOD, limit of detection.
[a] MCPP is the major metabolite of DnOP, but is not specific for DnOP.

Table 2.2
Bisphenols and other polychlorinated and phenolic substances.

Bisphenols	Abbreviation	LOD (ng/mL)
Bisphenol A (2,2-Bis(4-hydroxyphenyl) propane)	BPA	0.07
Bisphenol S (4,4′-Sulfonyldiphenol)	BPS	0.02
Bisphenol F (4,4′-Methylenediphenol)	BPF	0.05
1,1-Bis(4-hydroxyphenyl)ethane	BPE	0.06
2,2-Bis(4-hydroxy-3-methylphenyl)propane	BPC	0.06
2,2-Bis(4-hydroxy-3-isopropylphenyl)propane	BPG	0.03
1,1-Bis(4-hydroxyphenyl)-1,1diphenylmethane	BPBP	0.48
Other substances		
Triclosan		0.04
Triclocarban		0.34
Benzophenone-3		0.03
2,4-Dichlorophenol		0.04
2,5-Dichlorophenol		0.04
2,4,5-Trichlorophenol		0.01
2-Phenylphenol		0.04
4-Phenylphenol		0.11

Materials and Methods and Supplemental Tables 1–7.

Urine samples were measured in nine batches including 30–35 samples from the young men study for analysis of metabolites of the phthalates and substitutes and in six batches including about 50 samples from the young men study for analysis of the phenols. All batches also include calibration standards, three blanks, three urine pool controls and three urine pool controls spiked with a mixture of native phthalate, DEHTP and DINCH metabolite standards or a mixture of bisphenol and other phenol standards at low and high concentration levels.

2.3. Adjustment for urinary dilution

To account for urinary dilution of the chemicals or their metabolites above the LODs, all urinary concentrations were adjusted for individual urinary osmolality. This adjustment method was chosen because osmolality is less affected by other exogenous factors than other adjustment methods (Middleton et al., 2016). Osmolality was measured by the freezing point depression method with automatic cryoscopic osmometer (Osmomat ®030 from Gonotec, Berlin, Germany). Urinary osmolality ranged from 0.115 to 1.212 osm/kg with a mean value 0.863 osm/kg for all 300 urine samples. All measured urinary concentrations were subsequently normalized to the group median osmolality using the following equation:

$$\text{Osmolality adj. conc. (ng/mL)} = \frac{\text{urinary conc. (ng/mL)} \times 0.863\ \text{(osm/kg)}}{\text{sample osmolality (osm/kg)}}$$

Measured concentrations below LOD were registered as < LOD irrespective of the osmolality of the sample.

2.4. Statistical analysis

For several of the phthalates, DEHTP and DINCH urinary concentrations of more than one metabolite were measured. To simplify the data analysis, we summed the individual metabolites of each parent compound (DiBP, DnBP, DEHP, DHxP, DHpP, DiNP, DiDP, DEHTP, DINCH) instead of evaluating separately the concentrations of each metabolite measured. To sum up the metabolites for each phthalate, DEHTP and DINCH, the molar concentrations of the specific metabolites were calculated, summed and expressed in ng/ml by multiplying the molar sums with the molecular weight of their respective phthalate diester.

Descriptive statistics per study year for chemical concentrations are presented as the median, 75 and 95 percentiles and maximum concentrations. Summed phthalates, DEHTP and DINCH metabolites and all other measured substances were only included in additional analysis if they were detectable in ≥ 50% of the samples in each of the three study years examined.

For further statistical analyses all chemical concentrations below LOD were substituted by LOD/2. For individuals where all metabolites in a summed phthalate, DEHTP or DINCH were < LOD, the value was substituted with the lowest LOD/2. Since the chemical concentrations were not normally distributed, all data were normal logarithmically transformed to get an equal distribution. For analyses of correlations between the concentrations of the metabolites within summed phthalate, DEHTP and DINCH metabolites and in between the other phthalate metabolites (not expressed as sums), bisphenols and other phenols, bivariate correlation analyses (Spearman's rho (ρ)) were computed. To compare medians across the three selected study years two tailed Mann-Whitney U-tests were computed. To estimate the rate of changes over time, we conducted general linear regression analysis with the normal logarithmically transformed osmolality adjusted concentration of the individual chemicals or summed chemical metabolites as the dependent variable and the year of collection entered in as fixed categorical variable. Models were adjusted for BMI and age, which were included as continuous covariates. For estimation of average change per year, collection year, BMI and age, were all included in the model as continuous covariates. p-values < 0.05 were considered as statistically significant and p-values < 0.1 as border significant. Data analysis was performed using IBM SPSS Statistics 22 (IBM, New York, USA) and graphs were created using GraphPad Prism 8 Software.

3. Results and discussion

To our knowledge we here present the first Danish biomonitoring data on several new bisphenols, DEHTP and DINCH and some less studied phthalates such as DHxP and DHpP together with data on the more well-known phthalates, BPA and other phenols such as benzophenone-3, triclosan and some polychlorinated and phenolic substances. All urine samples were collected in 2009, 2013 and 2017 and thereby the data represent the exposure trends to these chemicals over the past decade.

3.1. Analytical method and validation

Limits of detection for the phthalate method ranged from 0.02 ng/mL (OH-MHpP) to 0.43 ng/mL (MEP) (Table 2.1), while LODs for substances included in the phenol method were ≤ 0.11 ng/mL except for BPBP (0.48 ng/mL) and triclocarban (0.34 ng/mL) (Table 2.2). Calibration curves for both methods spiked in a urine pool and in Milli-Q water were all linear in the measurement range with correlations coefficients (r^2) ≥ 0.98 (Supplemental Table 6.1 and 6.2).

The accuracy expressed as percent recovery for each analyte in all control material (Q low and Q high) was above ≥ 90% except Q low for BPF (84%) and Q low and Q high for triclocarban (87 and 89%, respectively). The intra-day precision expressed as relative standard deviation (RSD) for Q low and Q high were < 10% for all analytes except for MnOP, MiNP, MiDP and triclosan (all ≤ 16%) (Supplemental Table 6.1 and 6.2).

The inter-day precision (RSD) for control material Q low and Q high analyzed in all sample batches were ≤ 12% for all analytes in both the phthalate and the phenol method, except RSD for Q low or Q high for MMP, MnPrP, 3OH-MnBP, MnHxP, MnOP, MiNP, OH-MiDP, BPA, BPBP, triclosan and triclocarban which were ≤ 20% (Supplemental Table 7.1 and 7.2). Thus, the results of the very thorough validation procedure showed very good accuracy, precision and high sensitivity with low limits of detection for almost all the analytes evaluated in the two methods.

Table 3
Osmolality adjusted concentrations of phthalate metabolites (ng/mL) in urine collected in 2009, 2013 and 2017 from young Danish men.

Phthalate	Metabolite	2009 (n = 100)					2013 (n = 100)					2017 (n = 100)				
		n < LOD[a]	Median	75 P	95 P	Max.	n < LOD[a]	Median	75 P	95 P	Max.	n < LOD[a]	Median	75 P	95 P	Max.
DMP	MMP	8	2.63	5.01	10.5	99.3	11	2.39	3.68	6.76	18.5	19	1.83	2.91	6.67	30.1
DEP	MEP	0	77.6	233	1565	6255	0	25.4	54.6	430	812	0	23.9	55.9	283	1863
DiPrP	MiPrP	82		< LOD	0.24	8.86	87		< LOD	0.14	4.63	94		< LOD	0.08	0.32
DnPrP	MnPrP	89		< LOD	0.23	0.65	86		< LOD	0.39	0.81	92		< LOD	0.15	0.52
DiBP	MiBP	0	46.0	62.0	175	444	0	28.4	38.4	90.6	122	0	23.1	34.2	70.7	225
	2OH-MiBP	0	8.76	13.5	35.3	100	3	5.14	8.31	16.6	23.6	2	3.45	6.39	12.7	39.5
	ΣDiBPm	0	67.4	94.3	264	626	0	41.8	54.7	126	180	0	32.1	51.4	111	299
DnBP	MnBP	0	42.0	63.9	139	215	0	25.6	38.3	69.3	145	0	20.9	35.5	83.1	215
	3OH-MnBP	12	3.07	5.07	11.0	14.4	20	1.79	2.93	5.42	12.3	20	1.62	2.61	7.08	12.2
	ΣDnBPm	0	54.4	85.4	182	280	0	33.7	50.4	89.1	196	0	27.9	46.7	112	284
BBzP	MBzP	1	9.45	17.3	40.1	53.2	3	4.62	7.45	17.6	44.3	3	2.54	4.62	25.7	77.7
DnPeP	MnPeP	96			< LOD	4.02	98			< LOD	3.36	94		< LOD	0.16	2.29
DEHP	MEHP	0	3.15	5.43	11.6	35.0	1	1.92	2.97	7.18	19.6	3	1.09	1.94	4.57	9.85
	5OH-MEHP	0	15.9	23.4	63.5	126	0	8.97	12.4	23.9	179	0	5.64	8.32	27.7	51.1
	5oxo-MEHP	0	10.7	16.6	38.6	71.5	0	6.07	8.61	16.4	122	0	3.81	5.79	16.0	31.5
	5cx-MEPP	0	17.4	25.4	46.5	135	0	9.60	13.2	21.8	233	0	7.49	10.2	27.5	40.6
	2cx-MMPP	0	17.6	30.1	60.5	209	0	10.6	14.8	45.0	147	0	7.94	13.7	45.8	1012
	ΣDEHPm	0	85.0	130	320	747	0	51.3	73.3	130	910	0	34.7	57.1	189	1348
DHxP	MnHxP	35	0.09	0.24	0.84	2.78	30	0.08	0.19	0.41	0.99	41	0.07	0.22	1.34	2.44
	OH-MHxP	99			< LOD	0.47	98			< LOD	0.66	96			< LOD	0.35
	cx-MPeP	25	0.76	1.48	4.29	15.2	28	0.53	1.03	5.18	57.6	19	0.55	1.04	5.19	85.2
	ΣDHxPm	25	1.09	2.02	6.49	19.0	28	0.76	1.69	6.20	68.8	19	0.94	1.61	7.73	102
DCHP	MCHP	99			< LOD	1.40	100				< LOD	100				< LOD
DHpP	MnHpP	85		< LOD	0.26	1.10	81		< LOD	0.21	0.52	85		< LOD	0.10	0.66
	OH-MHpP	80		< LOD	0.79	1.14	97			< LOD	0.23	97			< LOD	0.47
	cx-MHxP	18	0.06	0.20	0.39	0.89	27	0.07	0.15	0.27	0.37	37	0.09	0.15	0.33	0.37
	ΣDHpPm	18	0.20	0.42	1.11	2.97	27	0.13	0.24	0.55	0.91	37	0.13	0.21	0.59	0.91
DnOP	MnOP	100				< LOD	99			< LOD	0.03	100				< LOD
	MCPP	1	1.18	2.00	4.20	7.25	1	0.88	1.37	3.43	56.5	2	0.75	1.20	3.58	77.2
DiNP	MiNP	12	0.90	1.77	4.31	6.54	13	0.63	1.15	4.70	14.5	19	0.44	0.81	2.58	100
	OH-MiNP	1	4.53	8.82	18.9	51.8	0	2.85	4.80	21.5	77.6	0	2.87	5.16	22.9	636
	oxo-MiNP	3	2.41	4.83	9.40	16.4	1	1.29	2.67	14.5	33.4	0	1.41	2.91	13.3	350
	cx-MiOP	0	9.41	14.4	43.6	89.0	0	5.24	9.04	37.8	127	0	4.06	7.14	58.2	675
	ΣDiNPm	0	23.5	39.8	84.3	212	0	13.3	25.3	103	337	0	12.5	22.8	136	2364
DiDP	MiDP	89		< LOD	0.45	13.4	97			< LOD	29.0	100				< LOD
	OH-MiDP	11	0.66	1.44	2.85	122	7	0.52	0.91	3.17	681	3	0.41	0.78	5.70	21.4
	oxo-MiDP	4	0.66	1.15	2.01	50.2	5	0.68	0.99	2.31	299	1	0.91	1.41	6.11	40.7
	cx-MiNP	7	0.90	1.45	4.04	65.7	7	0.66	1.06	3.10	724	8	0.36	0.60	2.54	7.64
	ΣDiDPm	4	3.33	5.31	10.8	326	5	2.86	4.63	11.9	2324	1	3.83	5.89	29.8	146

Σ, sums of phthalate metabolites were calculated by adding the molar concentrations of metabolites within the same phthalate, and then converted to concentrations in ng/mL by multiplying with the molecular weight of their respective parent compound.
P, percentile.
[a] n < LOD: concentrations below LOD were determined in urine before osmolality adjustment, data are shown in Supplemental Table 8.

3.2. Urinary excretion of phthalate metabolites

Medians, 75 and 95 percentiles and maximum levels of osmolality adjusted and un-adjusted urinary phthalate metabolite concentrations according to collection years are shown in Table 3 and Supplemental Table 8, respectively. One or more metabolites of seven (DEP, DiBP, DnBP, BBzP, DEHP, DiNP and DiDP) out of the 15 phthalate diesters analyzed were detectable above the limit of detection (LOD) in more than 95% of all samples irrespective of the sampling year. For four phthalates (DMP, DHxP, DHpP, and DnOP) at least one metabolite was above LOD in > 58% of the samples in all years of sampling (ranging from 59 to 81%). It should be noted that MCPP, a known metabolite of DnOP, but not specific for DnOP, was the only metabolite of the two DnOP metabolites that were detected in the majority of samples (MnOP was only measured in one out of the 300 samples). Thus, MCPP may arise from other phthalates, such as DnBP and several common high molecular weight phthalates like DiOP, DiNP and DiDP (Calafat et al., 2006). DiPrP, DnPrP, DnPeP, and DCHP were present above LOD in less than 20% of the samples (ranging from 0 to 18%). The observed low levels of the metabolites of DnPeP and DCHP are in accordance with previous studies (CDC, 2019; Koch et al., 2017; Rocha et al., 2017) and they will not be discussed further.

For eight of the phthalates (DMP, DEP, BBzP, DiBP, DnBP, DEHP, DHpP, DiNP) we observed significantly lower urinary concentrations of their metabolites in samples collected in the later study years (Table 3 and Fig. 1). In general, the largest decrease was observed from 2009 to 2013 and overall the decrease ranging from 36% for DiNP to 75% for MEP during the whole period (Supplemental Table 9). For DMP the detection rate of its metabolite MMP also slightly decreased from 92% in 2009 to 81% in 2017. Likewise, the detection rate of metabolites of DHpP decreased from 82% in 2009 to 67% in 2017. The urinary concentrations of MCPP were also significantly lower in the more recently collected samples (Table 3).

We have previously described the first indication on this decrease over time within the same population, based on our measurements of urinary concentrations of DEP, DnBP and DEHP metabolites in samples collected from young men (n = 881) in the period from 2007 to 2009 (Joensen et al., 2012). A similar significant decrease over time was observed for the urinary excretion of the metabolites of DMP, DEP, BBzP, DiBP, DnBP and DEHP in young Germans participating in the German Environmental Specimen Bank (ESB) from 1988 to 2015 (Koch et al., 2017), the metabolites of DEP, BBzP, DnBP and DEHP in Swedish women from 2009-14 (Gyllenhammar et al., 2017) and in general in US population groups participating from 1999 to 2016 in the NHANES studies (CDC, 2019). The urinary concentrations of MiBP decreased over time in both the German and Danish population but increased in the same period in the USA. While we in this Danish study observed significantly decreasing DiNP metabolites over time, no significant change was observed in the Swedish, German or US studies (CDC, 2019; Gyllenhammar et al., 2017; Koch et al., 2017). Finally, the decrease in the MCPP over time observed in this study was in accordance with other time trend studies (CDC, 2019; Koch et al., 2017) and possibly reflect decreasing exposure to DnOP and other common phthalates such as DnBP, DiOP and DiDP.

Similar median urinary concentrations were observed for the metabolites of DEHP in Denmark (2017), Germany (2015) and US (2015-16), while the urinary levels of both DiBP and DnBP metabolites were more than twice as high in Danish men compared to the levels in Germany and US. For DEP the metabolite concentrations in Danish and US men were similar but almost twice as high as in the Germans. For both the metabolites of DiNP and BBzP, the US population had the highest concentrations followed by the Danish young men; the Germans had the lowest concentrations (CDC, 2019; Koch et al., 2017).

In the present study, urinary concentrations of DiDP metabolites were generally low but slightly higher in the most recently collected samples (Table 3 and Fig. 1). It remains to be seen if this represents an emerging increase in DiDP exposure in the Danish population. No similar time trend was found for urinary DiDP metabolites in the comparable Swedish and German studies (Gyllenhammar et al., 2017; Koch et al., 2017).

No significant difference over time was observed for the urinary concentrations of the summed metabolites of DHxP (Table 3 and Fig. 1). Three urinary metabolites of DHxP were measured, but only the hydroxylated primary metabolite MnHxP and the secondary carboxylated metabolite cx-MPeP were detected in the majority of samples (≥ 59%). Even though only very low concentrations of MnHxP (< 0.1 ng/mL) were detected compared to the almost ten times higher concentrations of cx-MPeP, the concentration of the two metabolites were significantly correlated (Supplemental Table 10). To our knowledge correlation between urinary DHxP metabolites has not previously been reported. Another study recently measured MnHxP in Brazilian children (n = 300) and with almost similar detection limits (0.05 ng/mL) as ours, it was observed that only 3.3% of children excreted MnHxP in urine (Rocha et al., 2017). However, in that study no secondary metabolites of DHxP were measured and thereby the low number of samples with concentrations above LOD could not be confirmed by correlation to secondary metabolites, which according to our observation are likely to be present in higher concentrations and accordingly with a higher detection rate.

For all the phthalates (DiBP, DnBP, DEHP, DHxP, DiNP and DiDP) where two or more metabolites were measured in the majority of samples, the metabolites of each phthalate were highly correlated (Supplemental Table 10). The half-lives of the urinary metabolites of DiBP and DnBP, both primary and secondary metabolites, have been estimated to be almost the same (Koch et al., 2012), while for the high molecular weight phthalates, such as DEHP and DINP, the excretion rate for their respective primary monoesters (MEHP and MiNP) were much faster compared to the secondary metabolites especially the carboxylated metabolites (Koch and Angerer, 2007; Koch et al., 2006). Therefore, the high correlation coefficients between metabolites from each of the high molecular weight phthalates in the present study might confirm ongoing co-excretion of metabolites within the same phthalate and thereby indicating continuous exposure to these chemicals from relatively constant background sources.

3.3. Urinary excretion of DEHTP and DINCH metabolites

Table 4 and Supplemental Table 11 show the osmolality adjusted and un-adjusted urinary concentrations of DEHTP and DINCH metabolites. The number of participants excreting one or more metabolites of these phthalate substitutes increased over the period from 2009 to 2017 from 66 to 100% for DEHTP and from 81 to 98% for DINCH. Also, the urinary median concentrations of DINCH and DEHTP metabolites increased more than 2 and 20 times, respectively, from 2009 to 2017 (Table 4, Fig. 1 and Supplemental Table 9). Only few previous studies have reported changes in human urinary excretion of the DEHTP and DINCH metabolites.

The same DEHTP metabolites have been measured in urine samples from young German adults sampled in the German Environmental Specimen Bank (ESB) from 1999 to 2017 (Lessmann et al., 2019). Very similar to our findings, they report a rapid increase in DEHTP metabolite detection rates (8% in 2009, 95% in 2013 and 100% in 2017) with similar metabolite distributions (5cx-MEPTP being the major urinary DEHTP metabolite) and very similar, only slightly lower metabolite levels (median 5cx-MEPTP, un-adjusted, in 2013: 0.91 vs. 1.23 µg/L; 2017: 3.35 vs. 3.79 µg/L). Measurements of the two DEHTP metabolites; 5OH-MEHTP and 5cx-MEPTP have also been reported in the latest HBM study of the general US population (NHANES) including samples collected in 2015–2016 (CDC, 2019; Silva et al., 2019). Due to lower LODs for 5OH-MEHTP and 5cx-MEPTP in the present study, we found a higher detection rate of DEHTP metabolites already in 2009 compared to the US study (Silva et al., 2017). However, the median

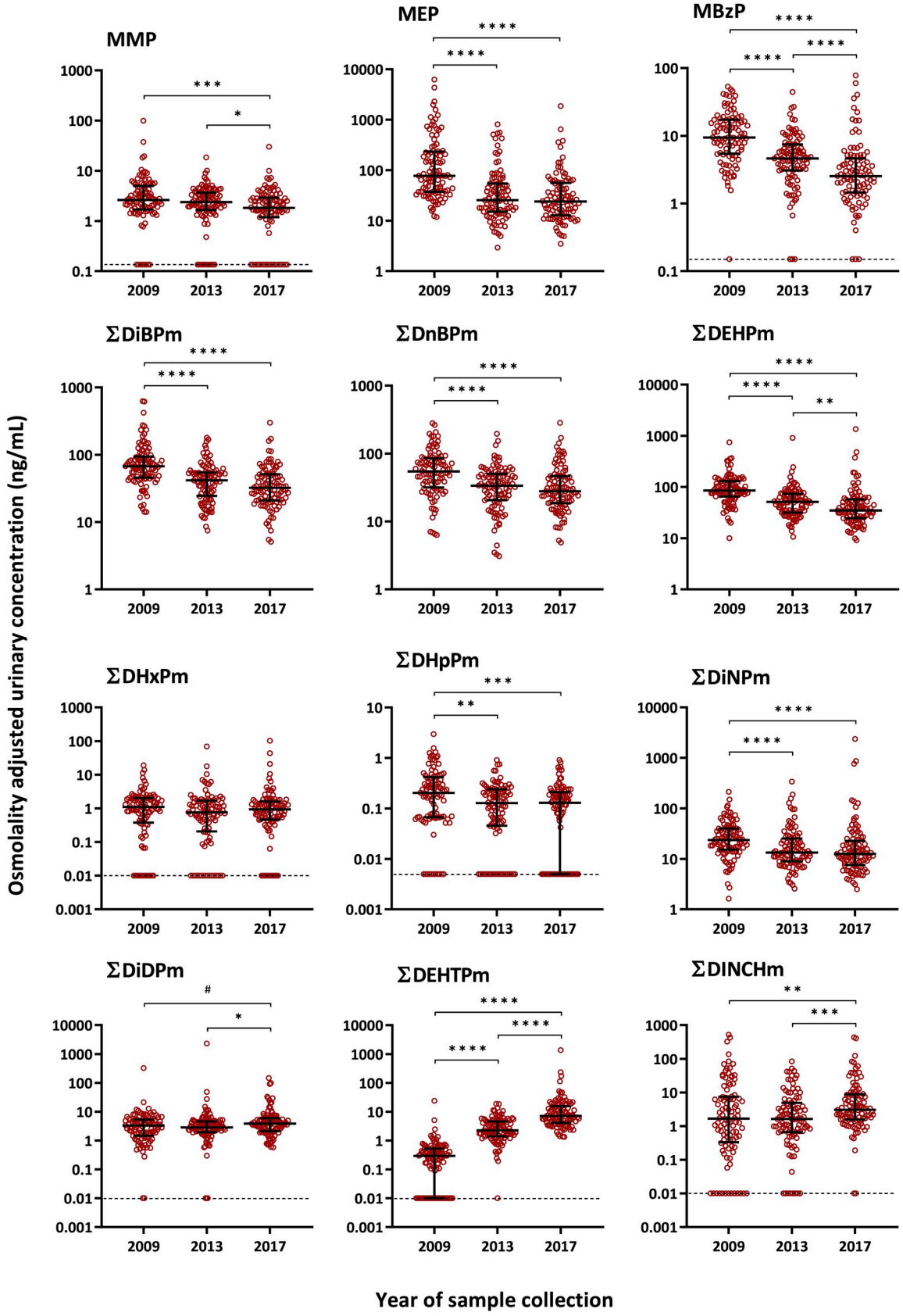

Fig. 1. Urinary osmolality adjusted concentration of phthalate metabolites, selected sums of phthalate metabolites and phthalate substitutes collected in 2009 (n = 100), 2013 (n = 100) and 2017 (n = 100. Bars represent median, 25 and 75 percentiles. Significant differences are considered as $p < 0.05$, where * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$ and # = $p < 0.1$ (border significant). Points on dotted lines were below the respective LODs and are illustrated as LOD divided by 2 (< LOD = LOD/2). All abbreviations are explained in Table 2.1 and combined metabolites in Table 3.

Table 4
Osmolality adjusted concentrations of phthalate substitute metabolites (ng/mL) in urine collected in 2009, 2013 and 2017 from young Danish men.

Phthalate substitute	Metabolite	2009 (n = 100)					2013 (n = 100)					2017 (n = 100)				
		n < LOD[a]	Median	75 P	95 P	Max.	n < LOD[a]	Median	75 P	95 P	Max.	n < LOD[a]	Median	75 P	95 P	Max.
DEHTP	5OH-MEHTP	98			< LOD	1.96	28	0.23	0.44	1.18	1.76	3	0.73	1.79	4.91	89.4
	5oxo-MEHTP	34	0.19	0.30	0.58	1.26	13	0.30	0.46	1.12	1.46	2	0.59	1.24	5.07	42.6
	5cx-MEHTP	77		< LOD	0.84	14.7	5	1.23	2.72	6.95	12.6	0	3.79	9.53	36.1	908
	2cx-MMHTP	97			< LOD	1.95	90		< LOD	0.34	0.80	69	< LOD	0.31	1.32	38.3
	ΣDEHTPm	34	0.29	0.53	1.47	23.9	5	2.25	4.65	10.8	18.7	0	7.10	15.6	51.0	1375
DINCH	OH-MINCH	23	0.80	3.20	42.5	198	13	0.80	2.29	19.8	41.9	2	1.56	4.13	39.7	238
	cx-MINCH	19	0.38	3.81	28.1	196	9	0.39	1.12	8.54	23.1	5	0.65	2.49	18.1	108
	ΣDINCHm	19	1.67	7.49	82.8	521	9	1.62	4.88	40.6	83.9	2	3.04	8.76	77.1	432

Σ, sums of DEHTP or DINCH metabolites were calculated by adding the molar concentrations of metabolites within the same compound, and then converted to concentrations in ng/mL by multiplying with the molecular weight of their respective parent compound.
P, percentile.
[a] n < LOD: concentrations below LOD were determined in urine before osmolality adjustment, data are shown in Supplemental Table 9.

urinary concentrations of 5OH-MEHTP and 5cx-MEPTP reported for US adult men in 2015-16 (CDC, 2019; Silva et al., 2019), were six and four times higher, respectively compared to the observed levels in our Danish men and young German adults in 2017. Another European study measured the same four DEHTP metabolites in urine collected in 2014-15 from 107 Portuguese children (Lessmann et al., 2017), and obtained concentrations similar to those we measured in 2017 samples from Danish men. Here, it must be taken into consideration that concentrations of the DEHTP metabolites in young children are in general higher than in adolescents and adults. This has also been demonstrated for a majority of the well-known phthalates in several previous studies (Frederiksen et al., 2014) and for DEHTP metabolites in the German pilot study and the US NHANES study (CDC, 2019; Lessmann et al., 2017; Silva et al., 2019).

In line with our study of excretion of DINCH metabolites, three other HBM time trend studies also observed similar increasing trends in urinary DINCH metabolite concentrations over time (Kasper-Sonnenberg et al., 2019), (Gyllenhammar et al., 2017) (CDC, 2019). Comparison between the median urinary concentrations of OH-MINCH and cx-MINCH in the present study and the German study of young adults participating in the German ESB from 1999 to 2017, showed almost similar ratios between OH-MINCH and cx-MINCH (3:1), but in 2017 the Danes had 2-3 times higher median concentrations of both metabolites than the Germans (Kasper-Sonnenberg et al., 2019). Median concentrations almost similar to our observations were measured in urine samples from 41 Portuguese adolescents collected in 2014-15 for both urinary OH-MINCH and cx-MINCH (Correia-Sa et al., 2017) and for OH-MINCH in urine collected in 2011–2012 from 208 German children in daycare centers (Fromme et al., 2016). However, as for the phthalate metabolites, concentrations of DINCH metabolites in children might be higher than in adults (Kasper-Sonnenberg et al., 2019). The latest reported HBM data from the NHANES study of the general US population on samples collected 2015-16 also included measurements of both OH-MINCH and cx-MINCH and observed a 1:1 ratio between the two metabolites in adults. The median concentration of cx-MINCH was almost similar in US and in the Danish men, while in contrast, in our 2017 samples the median OH-MINCH concentration was approximately 2.5 times higher than in US. Finally, the two DINCH metabolites were also reported in a Norwegian study using first morning urine samples collected from 61 adults in 2013-14; in that study lower amounts of both OH-MINCH and cx-MINCH were observed (Giovanoulis et al., 2016).

We observed strong and statistically significant correlations between the concentrations of metabolites of the same parent compound (e.g., DEHTP, DINCH) (Supplemental Table 10) as also reported in previous studies (Correia-Sa et al., 2017; Lessmann et al., 2017; Silva et al., 2013, 2019).

3.4. Changes and correlations between urinary excretion of phthalates and phthalate substitutes

The observed decreasing urinary excretion of metabolites of several of the phthalates such as DMP, DEP, DiBP, DnBP, BBzP, DHpP, DEHP and DiNP (Fig. 1) confirms that the overall exposure to these chemicals has decreased in Denmark during the past decade. Thus, for four of the phthalates DiBP, DnBP, BBzP and DEHP, this is consistent with a gradual phase out (EU_Commission_Regulation_phthlates, 2018). Despite the significant downward trends, in 2017, urinary concentrations of the metabolites of DiBP, DnBP, DEHP, DEP and DiNP were still the highest among all of the phthalates measured in the present study.

As suspected, the urinary concentrations of metabolites of the phthalate substitutes DEHTP and DINCH increased significantly over the studied period. Human kinetic studies have shown that the urinary excretion of the four DEHTP metabolites and the two DINCH metabolites only captures about 16.1% (Lessmann et al., 2016b) and 12.7% of the dose (Schutze et al., 2017), respectively within 48 h after dosing. On the other hand, 73.4% of DEHP dose were excreted within 48 h as the five DEHP metabolites MEHP, 5OH- and 5oxo-MEHP, 5cx- and 2cx-MEPP in a single man kinetic study (Koch et al., 2005), while another study including 20 participants observed lower excretion fractions for four of the five DEHP metabolites, ending up with a combined excretion fraction on 47.1% of dose (Anderson et al., 2011). When estimating exposure doses based on these different excretion fractions for DEHP, DEHTP and DINCH, the exposure to DEHP and DEHTP was almost similar in 2017, while the exposure to DINCH was estimated to be about two times lower. Thus, even though the increases in urinary excretion of the DEHTP and DINCH metabolites were far less than the observed decreases of the high molecular phthalate metabolites over the same period, a major part of the exposures to DEHP and other of the phthalates seems to have been substituted with DEHTP and DINCH. The further expanded EU restriction from 2020 on DiBP, DnBP, BBzP and DEHP, (EU_Commission_Regulation_phthlates, 2018), may be expected to lead to further decreases of the legacy phthalates. Therefore, it will be relevant to follow the time trend regarding the less well-known phthalates and the new substitutes such as DEHTP and DINCH.

Most of the phthalates and summed phthalate metabolites were significantly correlated, which confirms that humans are most likely exposed to several phthalates concurrently (Supplemental Table 12) as shown in previous studies (Frederiksen et al., 2013b; Hartmann et al., 2015). On the other hand, the summed DEHTP metabolites were negatively correlated with almost all the phthalates, except positively to the summed DiDP and DINCH metabolites. This is in accordance with the increasing urinary concentration of DEHTP, DINCH and DiDP over time compared to the decreasing time trends for the concentrations of the other phthalates.

Table 5
Osmolality adjusted Concentrations of bisphenols and other polychlorinated and phenolic substances (ng/mL) in urine collected in 2009, 2013 and 2017 from young Danish men.

	2009 (n = 100)					2013 (n = 100)					2017 (n = 100)				
	n < LOD[a]	Median	75 P	95 P	Max.	n < LOD[a]	Median	75 P	95 P	Max.	n < LOD[a]	Median	75 P	95 P	Max.
Bisphenols															
BPA	0	2.27	3.78	7.43	21.3	10	1.44	2.84	6.35	39.1	8	1.33	2.66	8.46	26.6
BPS	32	0.11	0.21	0.76	4.62	35	0.06	0.17	0.77	2.23	14	0.18	0.39	2.78	36.0
BPF	19	0.30	0.64	2.08	3.99	22	0.24	0.48	2.40	7.69	13	0.32	0.65	3.89	5.05
BPE	93		< LOD	0.16	1.73	92		< LOD	0.20	0.71	89		< LOD	0.35	1.29
BPC	80		< LOD	5.32	11.4	84		< LOD	4.11	19.6	81		< LOD	24.2	371
BPG	87		< LOD	4.25	9.54	90		< LOD	3.4	16.4	89		< LOD	20.3	311
BPBP	80			< LOD	0.06	85		< LOD	0.04	0.10	84		< LOD	0.02	0.07
Other substances															
Triclosan	1	3.63	25.4	356	1524	0	1.90	6.70	316	885	2	0.52	0.95	8.55	901
Triclocarban	100				< LOD	100				< LOD	99			< LOD	0.42
Benzophenone-3	0	3.79	9.21	111	1522	0	2.93	5.58	22.6	58.7	0	2.42	6.46	25.5	370
2,4-Dichlorophenol	1	0.45	0.80	3.16	8.34	7	0.26	0.43	1.72	3.10	5	0.21	0.31	0.66	1.97
2,5-Dichlorophenol	4	0.51	1.68	8.15	68.1	21	0.20	0.30	3.46	20.3	14	0.17	0.28	3.29	10.0
2,4,5-Trichlorophenol	43	0.04	0.10	0.22	0.35	43	0.03	0.07	0.21	0.73	41	0.03	0.05	0.08	0.33
2-Phenylphenol	13	0.13	0.21	0.46	1.01	4	0.13	0.19	0.36	0.50	12	0.11	0.15	0.45	3.40
4-Phenylphenol	6	0.71	1.52	5.39	13.8	9	0.61	1.23	2.83	3.08	22	0.35	0.57	3.05	19.5

P, percentile.
[a] n < LOD: concentrations below LOD were determined in urine before osmolality adjustment, data are shown in Supplemental Table 12.

3.5. Changes in urinary excretion of bisphenols

Medians, 75 and 95 percentiles and maximum levels of osmolality adjusted and measured urinary concentrations of the bisphenols, separated according to collection years are shown in Table 5 and Supplemental Table 13, respectively. The urinary median osmolality adjusted concentration of BPA significantly decreased with 57% in from 2009 to 2017 (Supplemental Table 9). Also, the detection rate of urinary BPA decreased slightly over time (Fig. 2 and Table 5). In contrast, the detection rate of both urinary BPS and BPF increased over the study period to 86% and 87%, respectively in samples collected in 2017. Similar decreasing time trends for the urinary BPA level were observed in Swedish women in the period 2009-14 (Gyllenhammar et al., 2017), in US adults (Ye et al., 2015) and in the general US population participating in the NHANES studies from 2003 to 2014 (CDC, 2019). In contrast BPF but not BPS increased significantly in the Swedish study, while BPS increased in the US study of adults (Gyllenhammar et al., 2017; Ye et al., 2015). The urinary median concentrations of BPA, BPS and BPF in the latest collected samples (2017) in this study were almost similar to levels observed in US adult participating in the NHANES studies from 2013 to 2014 (CDC, 2019; Lehmler et al., 2018). However, it should be noted that BPF has been reported to occur naturally in certain foods, e.g. mustard (Huang et al., 2019; Zoller et al., 2016). Interestingly, in the US high BPF exposure has been observed in the past, long before the substitution of BPA began. Therefore, it is impossible in these studies to clarify, how much of the current BPF exposure is from natural sources or from BPF used in consumer products as substitute for BPA.

Very weak but significantly positive correlations were observed between BPA, BPS and BPF, which may indicate co-exposure rather than substitution of BPA (Supplemental Table 14). While BPA was significantly correlated to all the other bisphenols and other phenols, BPS and BPF were only correlated to a few of the other phenols.

To our knowledge BPA and in general other bisphenols, chlorinated and phenolic substances have only been measured in limited groups of the European populations, e.g. in women and children. Several previous European HBM studies (more than five years ago) reported urinary BPA excretion in e.g. mother-child pairs and pregnant women (Casas et al., 2011; Covaci et al., 2015; Frederiksen et al., 2013b; Philippat et al., 2012). However, only very few studies report newer (within the recent five years) excretion levels for BPA, such as for children in the European HELIX study, where median concentrations in samples collected in 2014-15 in six different EU counties were at least twice as high as for young men in the present study (Casas et al., 2018; Haug et al., 2018). However, as for the phthalates, children were also in general higher exposed to BPA than adolescents (Frederiksen et al., 2013a).

For the four other bisphenols we analyzed, detection rates of less than 20% were observed irrespective of the study year (Table 5). However, for BPC and BPG the 95 percentile and the maximum concentrations were much higher in the 2017 samples compared to both 2009 and 2013, which may indicate emerging sporadic source(s) of exposure to these two bisphenols. Future HBM studies are needed to confirm if this is a trend. To our knowledge, very few studies have reported HBM data on BPA analogues, and only BPS, BPF and bisphenol AF (BPAF) seem to be detectable above limits of detection (Chen et al., 2016). Recent European HBM data for BPA analogues is lacking and in that respect our study is timely.

Although the concentration of BPS increased in the past decade and BPF increased from 2013 to 2017, the combined increase of BPS and BPF was less than the concurrent decrease of BPA. Thereby BPS, BPF and other BPA analogues (which we only detected in few participants), did only partly seems to substitute BPA, which in our opinion is a positive development in the attempt to avoid BPA (EU_Commission_Regulation_BPA, 2018) and BPA analogues with suspected endocrine disrupting effects. However other substitutes to BPA such as bisphenol A diglycidyl ether (BADGE) remains to be analyzed in Danes and in several other populations.

3.6. Changes in urinary excretion of polychlorinated and phenolic substances

Osmolality adjusted and measured urinary concentrations of the analyzed polychlorinated and phenolic substances stratified by collection year are shown as medians, 75 and 95 percentiles and maximum levels in Table 5 and Supplemental Table 13, respectively.

Almost all our participants excreted triclosan in their urine, however, the median concentration of triclosan significantly decreased over the past decade with the 2017 median level being 89% lower compared to the 2009 level (Supplemental Table 9). The Swedish HBM trend study of women collecting urine samples from 2009-14 and the NHANES studies measuring triclosan in the period from 2003 to 14 showed similar decreasing time trend for triclosan (CDC, 2019;

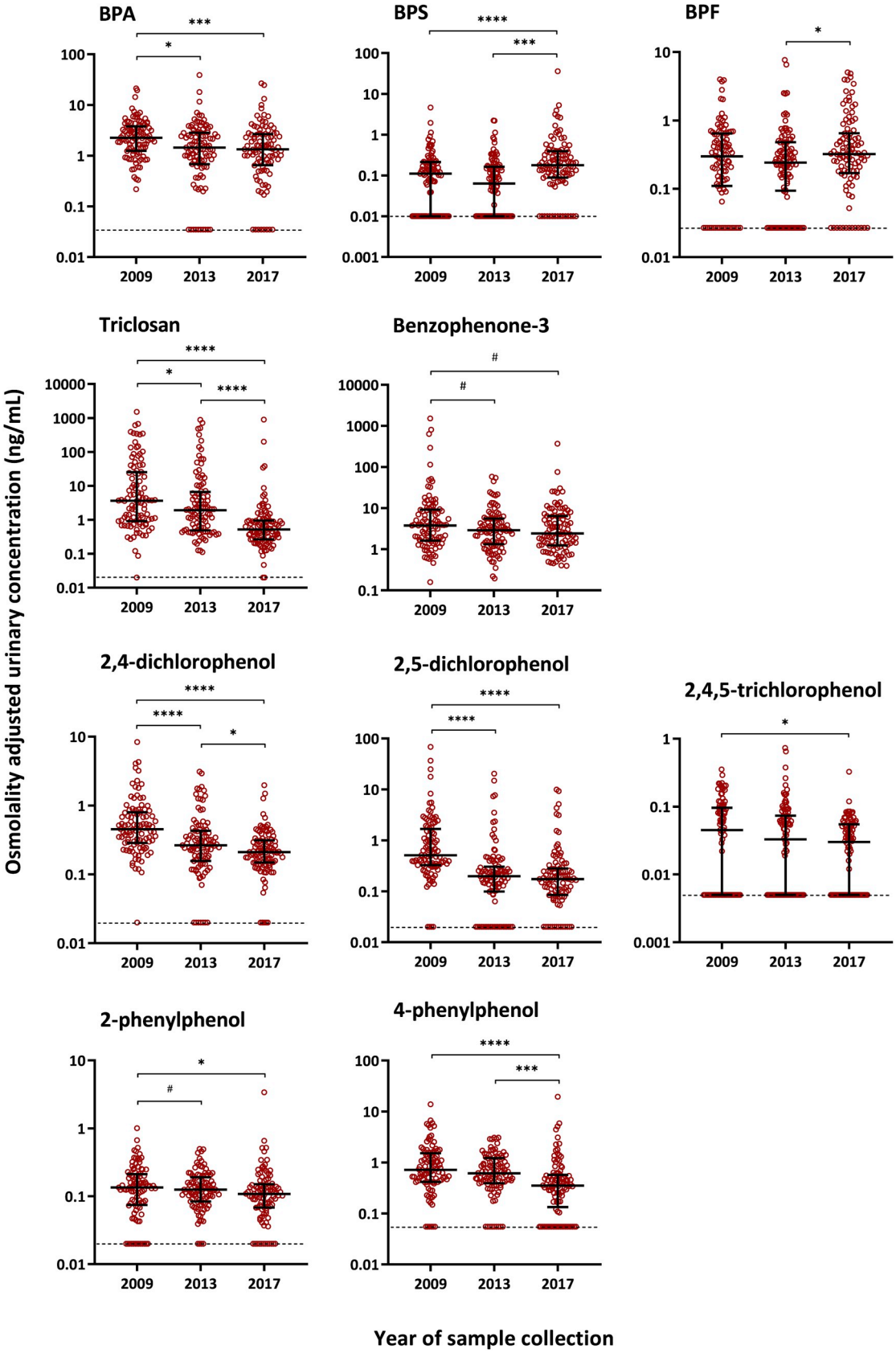

Fig. 2. Urinary osmolality adjusted concentration of bisphenol A (BPA), bisphenol S (BPS), bisphenol F (BPF), chlorinated and phenolic substances collected in 2009 (n = 100), 2013 (n = 100) and 2017 (n = 100). Bars represent median, 25 and 75 percentiles. Significant differences are considered as $p < 0.05$, where * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$ and # = $p < 0.1$ (border significant). Points on dotted lines were below the respective LODs and are illustrated as LOD divided by 2 (< LOD = LOD/2).

Gyllenhammar et al., 2017). However, the urinary median triclosan concentration among young men participating in this study were for 2013 and 2017 about three and ten times lower compared to the concentration in adult US men participating in the NHANES studies from 2013-14 (CDC, 2019). None of our participants, except one in 2017, excreted triclocarban This is in accordance with the NHANES studies, where only few participants excreted triclocarban and in contrast to pregnant women in Northern Puerto Rico, where more than 90% of the study group excreted triclocarban (CDC, 2019; Ashrap et al., 2018).

Benzophenone-3 was detected in all samples in the present study in line with our previous studies of different population groups (Frederiksen et al., 2014; Krause et al., 2016, 2018a). Here we observed a trend, albeit statistically non-significant, towards lower concentrations in more recently collected samples. Only the NHANES study was useful for comparison of time trends, but no change in exposure was observed for benzophenone-3 (CDC, 2019) and the latest reported median urine concentration of benzophenone-3 for US men in samples collected 2013-14 was more than five times higher compared to the Danish 2017 level in this study.

For comparison of the concentrations observed for triclosan and benzophenone-3 among the young men in our study and other European citizens, only the European HELIX study reported newer excretion levels in children (Casas et al., 2018; Haug et al., 2018). The Danish median concentrations for both triclosan and benzophenone-3 were within the levels observed in children in six different EU counties.

In Denmark triclosan has in several years only been allowed in very few personal products, such as toothpaste and deodorants and only in concentration ≤ 0.3%. Furthermore, since 2015 the use of triclosan was also banned in a broad range of disinfecting biocidal products to avoid accumulation in the aquatic environment. These gradual restriction might have caused the decrease in the exposure to triclosan (MST_Triclosan, 2019).

According to European legislation benzophenone-3 was until recently allowed for use as UV filter in concentration ≤ 10% in all cosmetic products, but from September 2017, benzophenone-3 use was restricted to ≤ 6% in sunscreen products and max 0.5% in all other cosmetic products (EU_Commision_Regulation_BP3, 2017). Benzophenone-3 is, however, still used as UV filter in many other consumer products, which might explain why benzophenone-3 only slightly decreased by time among the participant in this study.

From 2009 to 2017 more than 60% of our study participants excreted 2,4,5-trichlorophenol and most of the participants (> 78%) excreted 2,4-dichlorophenol, 2,5-dichlorophenol, 2-phenylphenol and 4-phenylphenol in their urine. Although, the urinary concentrations of the chlorinated- and phenylphenols were relatively low already in the 2009 samples they all decreased over time, ranging from 14% for 2-phenyl phenol to 80% for 2,5-dichlorophenol (Table 5, Fig. 2 and Supplemental Table 9). Similar time trends were also observed for 2,4-dichlorophenol and 2,5-dichlorophenol in the NHANES studies (CDC, 2019), although the urinary median concentrations in US men (2013-14) were more than two times higher and about ten times higher, respectively, compared to this study. In general, it seems like populations in several Asian countries, Brazilian and US are more exposed to 2,4-dichlorophenol and 2,5-dichlorophenol or their precursors than Europeans. However, only few studies have been conducted in Europe (Honda and Kannan, 2018; Rocha et al., 2018; Vernet et al., 2018).

The highest significant correlations were observed between triclosan and 2,4-dichlorophenol; 2,4-dichlorophenol and 2,5-dichlorophenol and 2-phenylphenol and 4-phenylphenol, which indicates co-exposure of these substances or their precursors (Supplemental Table 14).

As mentioned, the chloro- and phenylphenols are used, for example, as pesticides and fungicides in conventional agriculture. However, in Denmark the production of organic vegetables and fruit doubled over the period from 2015-17, as well as imported organic goods in general increased more than twice from 2012 to 2017 (Statistic Denmark, 2019). Thus, Denmark has during the last decade experienced a large increase in the sale of organic green groceries facilitated both by easier access and cheaper organic products. This development may explain the decreasing exposure to pesticide and fungicide contaminants such as the chloro- and phenylphenols.

4. Concluding remarks

Overall it is in our opinion, a positive development that the exposures to many of the chemicals evaluated in this study have decreased significantly during the past decade. On the other hand, this HBM time trend study included a convenience group of young men all living in an urban area, although these men were recruited among the general population. New HBM studies evaluating exposure patterns in the same age group of young men living in rural areas of Denmark or representing other subgroups of the population, such as pregnant, children and adolescents remain to be elucidated. Differences in exposures among age groups might be expected as shown in NHANES (CDC, 2019).

Furthermore, only very few other studies report time trend data on the most common of the here presented chemicals. Finally, humans are exposed to thousands of other industrial chemicals, many of which are suspected of having endocrine disrupting effects such as e.g. the BPA analogues bisphenol A diglycidyl ether (BADGE) and bisphenol AF (BPAF) (Desdoits-Lethimonier et al., 2017; Zhao et al., 2019) and the benzophenone-3 analogues benzophenone-2 and 4-hydroxybenzophenone (Buck Louis et al., 2014; Krause et al., 2018b). Therefore, since humans are still exposed to the here presented chemicals and to increasing amounts of their respective less known substitutes and analogues, the changes in recent exposures should be followed also in future investigations in different populations and counties. Furthermore, we do not know much about the sources for exposure to these chemicals, e.g. the less known bisphenols and phthalates and to e.g. triclosan and some of the pesticides although these chemicals almost should have been out phased from consumer products now. Therefore, source tracking must also be an important issue in future research and national control programs.

In conclusion, the significant decrease in internal exposure to most of the common and critical phthalates and BPA observed during the past decade may reflect the regulatory interventions that have been implemented in EU as well as voluntary market changes. Furthermore, significant decreasing exposure to triclosan and some chloro- and phenylphenols and partly to benzophenone-3 reflect a positive development, which might benefit people's health. Despite increasing exposures to the phthalate substitutes and BPA analogues, the combined concentrations of original and substitute chemicals showed a downward trend with time. These findings suggest only partial direct substitution, substitution by chemicals not covered in this study, or a general decline in the exposure to these all of these chemical/product groups over the last decade.

Funding

This study was supported by the Danish Environmental Protection Agency (j.nr.MST-611-00012), Denmark as a project under Centre on Endocrine Disrupters (http://www.cend.dk) and the International Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health (EDMaRC). The instrumental equipment was financially supported by Velux Fondene, Denmark; Augustinus Fonden, Denmark and Svend Andersen Fonden, Denmark. The cohort study of the young Danish men was funded by the European Commission (DEER; FP7-2007-212844), European Union; the Danish Ministry of Health, Denmark and the Danish Environmental Protection Agency, Denmark.

Declaration of competing interest

The findings and conclusions in the present study are those of the authors and do not necessarily represent the views of the funding sources. The authors' freedom to design, conduct, interpret, and publish research was not compromised by any controlling sponsor. The authors declare they have no actual or potential competing financial interests or other conflicts of interest.

Acknowledgements

We would like to thank the young men participating and the staff involved in different parts of the project including the recruitment procedures, physical examinations, collection and analysis of samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2019.10.002.

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International Journal of Hygiene and Environmental Health

doi:10.1016/S1438-4639(19)31038-7

International Journal of Hygiene and Environmental Health 223 (2020) 267–280

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

Evaluation of human biomonitoring data in a health risk based context: An updated analysis of population level data from the Canadian Health Measures Survey

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ARTICLE INFO

Keywords:
Biomonitoring
Environmental chemicals
Biomonitoring equivalents
CHMS
Hazard quotient
Cancer risk

ABSTRACT

In order to characterize exposure of the Canadian population to environmental chemicals, a human biomonitoring component has been included in the Canadian Health Measures Survey (CHMS). This nationally-representative survey, launched in 2007 by the Government of Canada, has measured over 250 chemicals in approximately 30,000 Canadians during the last decade. The capacity to interpret these data at the population level in a health risk context is gradually improving with the development of biomonitoring screening values, such as biomonitoring equivalents (BE) and human biomonitoring (HBM) values. This study evaluates recent population level biomonitoring data from the CHMS in a health risk context using biomonitoring screening values. Nationally representative biomonitoring data for fluoride, selenium, molybdenum, arsenic, silver, thallium, cyfluthrin, 2,4-dichlorophenoxyacetic acid (2,4-D), 3-phenoxybenzoic acid (3-PBA), chlorpyrifos, deltamethrin, bisphenol A, triclosan, acrylamide, cadmium, perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), bromoform, chloroform, benzene, toluene, xylene, ethylbenzene, styrene and tetrachloroethylene were screened as part as this study. For non-cancer endpoints, hazard quotients (HQs) were calculated as the ratio of population level concentrations of a specific chemical at the geometric mean and 95th percentile to the corresponding biomonitoring screening value. Cancer risks were calculated at the 5th, 25th, 50th, 75th and 95th percentiles of the population concentration using BEs based on a risk specific dose. Most of the chemicals analyzed had HQs below 1 suggesting that levels of exposure to these chemicals are not a concern at the population level. However, HQs exceeded 1 in smokers for cadmium, acrylamide and benzene, as well as in the general population for inorganic arsenic, PFOS and PFOA, 3-PBA and fluoride. Furthermore, cancer risks for inorganic arsenic, acrylamide, and benzene at most population percentiles of exposure were elevated ($> 10^{-5}$). Specifically, for inorganic arsenic in the general population, the HQ was 3.13 at the 95th percentile concentration and the cancer risk was 3.4×10^{-4} at the 50th percentile of population concentrations. These results suggest that the levels of exposure in the Canadian population to some of the environmental chemicals assessed might be of concern. The results of this screening exercise support the findings of previous risk assessments and ongoing efforts to reduce risks from exposure to chemicals evaluated as part of this study. Although paucity of biomonitoring screening values for several environmental contaminants may be a limitation to this approach, our assessment contributes to the prioritization of a number of chemicals measured as part of CHMS for follow-up activities such as more detailed characterization of exposure sources.

1. Introduction

Exposure of the general population to potentially harmful environmental chemicals is a growing global concern. Biomonitoring, the direct measurement of a biomarker (a chemical or its metabolites) in biological samples such as blood or urine, is an approach used to

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https://doi.org/10.1016/j.ijheh.2019.07.009
Received 16 May 2019; Received in revised form 19 July 2019; Accepted 20 July 2019

measure exposures to environmental chemicals (Angerer et al., 2011; Hays and Aylward, 2009). Human biomonitoring is included as a component of the Canadian Health Measures Survey (CHMS) to address the monitoring and surveillance component of the Government of Canada's Chemicals Management Plan (Canada, 2006a; Eykelbosh et al., 2018). The CHMS is an ongoing nationally representative survey initiated in 2007 by Statistics Canada in partnership with Health Canada and the Public Health Agency of Canada. This survey collects health and wellness data on the general population, such as weight, height, physical fitness and markers of chronic and infectious disease (Eykelbosh et al., 2018; Haines et al., 2017). The biomonitoring component of the CHMS measures environmental chemicals in blood, urine and/or hair (Health Canada, 2017c). The biomonitoring data have been published as summary statistics for different age and sex groups in CHMS biomonitoring reports for cycle 1 (2007–2009), cycle 2 (2009–2011), cycle 3 (2012–2013) and cycle 4 (2014–2015) (Health Canada, 2010c, 2013, 2015b; 2017c). Collectively, the first four cycles of the CHMS have provided population-level data for over 250 environmental chemicals in Canadians aged 3–79 years.

Biomonitoring data are of significant value in exposure assessments as they provide evidence of internal exposure to a chemical from all sources, routes and pathways (Angerer et al., 2011; Clewell et al., 2008; Sexton et al., 2004). However, a lack of appropriate health-based screening values for the general population may impede the interpretation of these biological measures in a health risk context (Hays et al., 2007). Health-based screening values are derived from epidemiological data demonstrating a direct, quantitative relationship between biomarker measurement and adverse health effects. Indeed, only a few substances (e.g. lead and mercury) have these health-based screening values because their development is highly resource and time intensive (Hays and Aylward, 2009). Consequently, the biomonitoring equivalent (BE) approach was developed by Hays et al. (2007) as an interim tool for interpreting population level biomonitoring data in a health risk context (Hays et al., 2007). A BE is the concentration of a biomarker of exposure for an environmental chemical in a biological medium (e.g. blood, urine) consistent with an existing exposure guidance value for that chemical (Hays et al., 2008a; LaKind et al., 2008). Exposure guidance values for non-cancer health effects include reference doses (RfD) from the U.S. Environmental Protection Agency (US EPA) and tolerable or acceptable daily intakes (TDI or ADI) from Health Canada. Cancer-based exposure guidance values include the risk-specific dose (e.g. dose associated with a 10^{-4} cancer risk from Health Canada) based on oral cancer slope factor or inhalation unit risk derived for different chemicals. This study also utilized other screening values such as human biomonitoring-I (HBM-I) values from the German Human Biomonitoring Commission for thallium, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) and biomonitoring guidance values (BGVs) for chlorpyrifos (Apel et al., 2017; Arnold et al., 2015; Steckling et al., 2018). HBM-I values are derived based on epidemiological data on human toxicity and, more recently, using internationally accepted TDI/RfD values or toxicologically well-founded points of departure observed in animal studies (Apel et al., 2017; Schulz et al., 2011). The BGVs for chlorpyrifos are not based upon existing exposure guidance values, but rather use physiologically based pharmacokinetic (PBPK) and pharmacodynamic models to predict levels of biomarkers that are an early indicator of adverse health effects (Arnold et al., 2015). In this article, we use the terminology “biomonitoring screening values” to encompass all of these values (BE, HBM-I, BGV). Biomonitoring screening values are tools that can be used for rapid screening of chemical biomonitoring data to identify exposures of concern and can contribute to ranking of chemical priorities for risk assessment and evaluation of risk management actions.

Previously, St-Amand et al. (2014) screened the CHMS population-level data from 2007 to 2009 and 2009–2011 in a risk-based context using BE values. The study suggested that exposures to most of the environmental chemicals assessed, except for inorganic arsenic and cadmium, were below existing exposure guidance values. Since this initial study, additional cycles (2012–2013, 2014–2015) of CHMS data have been released providing more recent biomonitoring data for a number of chemicals included in St-Amand et al. (2014), and data for additional chemicals not measured in earlier cycles. New or updated exposure guidance values and/or biomonitoring screening values have also recently been published for some of the chemicals measured as part of the earlier cycles of CHMS (e.g., silver, fluoride, PFOS, and PFOA). Consequently, this study aims to provide an updated interpretation of population level CHMS biomonitoring data in a health risk based context using a set of biomonitoring screening values in order to identify chemicals for which current levels of exposure could be a concern.

2. Methods

The following sections describe the criteria used in the selection of CHMS biomonitoring data for analysis, relevant biomonitoring screening values and, finally, the approach used in the risk-based screening of population-level biomonitoring data.

2.1. Selection of biomonitoring data

Biomonitoring data from the CHMS are representative of the general population aged 6–79 years for 2007–2009, and 3–79 years for 2009–2011, 2012–2013 and 2014–2015. An overview of the biomonitoring component of the CHMS as well as a complete list of chemicals measured between 2007 and 2015 are provided in Haines et al. (2017). All chemicals measured in the most recent cycle (2014–2015) with an existing biomonitoring screening value were included in this study, except for bromoform and tetrachloroethylene for which the cycle 3 (2012–2013) data were used due to a low detection rate or high coefficient of variation (CV) in Cycle 4. Chemicals measured in cycle 1 or 2 only and for which new biomonitoring screening values have been published since 2014 or for which an updated BE could be calculated based on a new exposure guidance value were also included in this study. These chemicals include cyfluthrin, deltamethrin, molybdenum, selenium, silver, thallium, 2,4-dichlorophenoxyacetic acid (2,4-D), 3-phenoxybenzoic acid (3-PBA), PFOS and PFOA.

Biomonitoring screening values developed for non-cancer endpoints were compared to the geometric mean (GM) and 95th percentile (P95) concentrations from the CHMS for the general Canadian population (Tables 1–3) (Health Canada, 2013, 2015b; 2017c). For some chemicals, evaluation was conducted using age-specific data, when screening values were derived for specific age groups (fluoride and chlorpyrifos) or when bioaccumulation with age is expected due to long elimination half-lives of the biomarkers (cadmium, PFOA and PFOA). For some chemicals, evaluations were conducted for smokers and non-smokers when evidence shows impact of smoking on blood or urinary concentrations as in the cases of acrylamide, cadmium, toluene, benzene, ethylbenzene, xylene and styrene (Health Canada, 2017c; Kirman et al., 2012). Statistical estimates for subpopulation of smokers and non-smokers as well as specific age-groups not available as part as CHMS biomonitoring reports were calculated *de novo* for this exercise. For calculation of cancer risk estimates, population percentiles (P5, P25, P50, P75, and P95) were calculated for smokers and non-smokers for benzene and acrylamide in blood, and for the total population for inorganic arsenic in urine (Table 4). Cancer risk analysis by smoking status was carried out for benzene and acrylamide based on evidence that biomonitoring data for these chemicals may be impacted by smoking (Health Canada, 2017c; Kirman et al., 2012). A urinary cotinine concentration cut-off of 50 ng/ml was used to define smokers ($\geq$50 ng/ml) and non-smokers ($<$ 50 ng/ml) (SRNT, 2002). The glycidamide haemoglobin adduct (GAVal) biomarker may be more critical than the parent compound for carcinogenic properties and, therefore, was used to assess cancer risks associated with exposure of acrylamide (EPA, 2010). For the purpose of this analysis, concentration of

Table 1
Non-persistent environmental chemicals: biomarkers, exposure guidance values, corresponding biomonitoring screening values and biomonitoring data from the Canadian Health Measures Survey.

Chemical group	Chemical (Biomarkers if different)	Exposure guidance values (type; reference)	Biomonitoring screening value, value, reference	Unit and matrix	CHMS data				
					Cycle (years)	Age group years	n	GM (95% CI)	P95 (95% CI)
Metals and trace elements	Arsenic, inorganic (sum iAs, DMA, MMA)	0.0003 mg/kg/d (RfD; US EPA, 1991a)	BE: 6.4 Hays et al. (2010)	µg As/L in urine	Cycle 4 (2014–2015)	3–79	2567	5.1 (4.5–5.6)	20 (15–25)
	Fluoride	0.105 mg/kg/d (TDI; Health Canada, 2010b)	BE: 3–6 years:1.36 6–10 years:1.5 10–18 years: 2.5 Aylward et al. (2015)	µg/L in urine	Cycle 4 (2014–2015)	3-5 6-11 12-19 20-39 40-59 60–79	483 533 481 369 368 340	0.42 (0.33–0.54) 0.47 (0.37–0.6) 0.42 (0.35–0.5) 0.46 (0.35–0.6) 0.49 (0.36–0.66) 0.51 (0.43–0.61)	1.6[a] (0.66–2.5) 1.6 (1.1–2) 1.1 (0.91–1.2) 1.4 (1.1–1.7) 1.4 (1.1–1.7) 1.8 (1.2–2.4)
	Molybdenum	0.17 mg/kg/d (NOAEL + UF; Health Canada, 2016)	BE: 7516[b] Hays et al. (2016)	µg/L in urine	Cycle 2 (2009–2011)	3–79	5738	45 (42–48)	170 (150–190)
	Selenium	400 µg/L (UL; IOM, 2000)	BE: 480 Hays et al. (2014)	µg/L in whole blood	Cycle 2 (2009–2011)	6–79	5575	190 (190–190)	240 (230–250)
	Silver	0,005 mg/kg/d (RfD; US EPA, 1991b)	BE: 0.4 Aylward et al. (2016)	µg/L in whole blood	Cycle 2 (2009–2011)	20–79	3617	0.070 (0.056–0.086)	0.30 (0.25–0.35)
	Thallium	NA	HBM-I: 5 Apel et al. (2017)	µg/L in urine	Cycle 2 (2009–2011)	3–79	6311	0.23 (0.21–0.24)	0.62 (0.55–0.70)
Pesticides	Cyfluthrin (4-F-3-PBA)	0.005 mg/kg/d (ADI; Health Canada, 2017g)	BE: 46[c] Hays et al. (2009)	µg/L in urine	Cycle 2 (2009–2011)	3–79	2022	NA[d]	0.11[a] (0.040–0.17)
	Deltamethrin (cis-DBCA)	0.003 mg/kg/d (ADI; Health Canada, 2015a)	BE: 20[e] Aylward et al. (2011)	µg/L in urine	Cycle 2 (2009–2011)	3–79	2535	0.012 (0.010–0.014)	0.15 (0.073–0.23)
	2,4-Dichlorophenoxyacetic acid	0.21 mg/kg/d (RfD; US EPA, 2016)	BE: 3–6 years: 7000[f] 15 years +: 10 500[f] Aylward and Hays (2015)	µg/L in urine	Cycle 2 (2009–2011)	3-5 6–79	523 2028	0.26 (0.23–0.30) NA[d]	1.1 (0.81–1.4) 1 (0.86, 1.2)
	Pyrethroid pesticide, various (3-PBA)	0.001–0.25 mg/kg/d (US EPA RfD; see reference in Aylward et al., 2018)	BE: 1.7 (tier 1) 87 (tier 2) Aylward et al. (2018)	µg/L urine	Cycle 2 (2009–2011)	3–79	1994	0.43 (0.34–0.53)	5.9[a] (2.2–9.5)
	Chlorpyrifos (TCPy)	NA	BGV: 520 - Infant 2100 - adult Arnold et al. (2015)	µg/L urine	Cycle 4 (2014–2015)	3-5 6-11 12-19 20-39 40-59 60–79	479 489 478 336 299 341	1.3 (1.1–1.5) 1.6 (1.3–2.1) 1.5 (1.3–1.7) 1.3 (1.1–1.5) 1.3 (1.1–1.7) 1.4 (1.2–1.7)	7.3[a] (4.5–10) NA[g] 11[a] (6.3–15) 8.4 (5.9–11) NA[g] 9.7[a] (3.8–16)
Environmental phenols	Bisphenol A	0.025 mg/kg/d (pTDI; Health Canada, 2008)	BE: 1000 Krishnan et al. (2010a)	µg/L urine	Cycle 4 (2014–2015)	3–79	2049	0.93 (0.87–0.99)	4.5 (3.9–5.2)
	Triclosan	0.08 mg/kg/d (ADI; ECCC and HC, 2016)	BE: 1783[h] Krishnan et al. (2010b)	µg/L urine	Cycle 4 (2014–2015)	3–79	2047	NA[d]	660[a] (370–940)

(*continued on next page*)

Table 1 (*continued*)

Chemical group	Chemical (Biomarkers if different)	Exposure guidance values (type; reference)	Biomonitoring screening value, value, reference	Unit and matrix	CHMS data				
					Cycle (years)	Age group years	n	GM (95% CI)	P95 (95% CI)
Acrylamide	Acrylamide (AAVal)	0.002 mg/kg/d (RfD; US EPA, 2010)	BE: 190 Hays and Aylward (2008); Aylward et al. (2013)	pmol/g Hb, whole blood	Cycle 4 (2014–2015)	3–79 (NS)	2187	56 (51–61)	110 (81–140)
						3–79 (S)	265	130 (110–140)	290 (220–360)
	Acrylamide (GAVal)	0.002 mg/kg/d (RfD; US EPA, 2010)	BE: 176[i] Hays and Aylward (2008)	pmol/g Hb, whole blood	Cycle 4 (2014–2015)	3–79 (NS)	2187	52 (47–58)	110 (93–130)
						3–79 (S)	265	100 (87–110)	250 (180–320)

Abbreviations: 2,4-D: 2,4-Dichlorophenoxyacetic acid, 3-PBA: 3-Phenoxybenzoic acid, 4-F-3-PBA: 4-Fluoro-3-phenoxybenzoic acid, AAVal: Acrylamide hemoglobin valine terminal adducts, ADI: Acceptable daily intake, BE: Biomonitoring equivalent, BGV: Biomonitoring guidance values, CHMS: Canadian Health Measures Survey, CI: Confidence interval, cis-DBCA, cis-3-(2,2-Dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid, DMA: Dimethylarsinic acid, GAVal: Glycidamide hemoglobin valine terminal adducts, GM: Geometric mean, Hb: Hemoglobin, HBM-I: Human biomonitoring value I, iAS: Inorganic arsenic, IOM: Institute of Medecine; MMA: Monomethylarsonic acid, NA: Not available, NS: Non-smokers, P95: 95th percentile, POD: Point of departure, pTDI: Provisionnal tolerable daily intake, RfD: Reference dose, S: Smokers, TCPy: 3,5,6-Trichloro-2-pyridinol, TDI: Tolerable daily intake, UF: Uncertain factor, UL: Tolerable upper intake levels.

[a] Data should be used with caution as the coefficient of variation is between 16.6% and 33.3%.

[b] A new BE was calculated using a recent NOAEL adopted by Health Canada (Health Canada, 2016), methodology as described previously (Hays et al., 2016), and a UF of 100.

[c] A new BE was calculated using a recent Health Canada ADI (Health Canada, 2017g), methodology as described previously (Hays et al., 2009), and a UF of 300 applied to the POD.

[d] If > 40% of samples were below the limit of detection, the GM was not calculated.

[e] A new BE was calculated using a recent Health Canada ADI (Health Canada, 2015a), methodology as described previously (Aylward et al., 2011), and a UF of 300 applied to the POD.

[f] The BE was updated since the previous CHMS screening analysis (St-Amand et al., 2014) to reflect changes made to the US EPA assessment.

[g] Data are too unreliable to be published.

[h] A new BE was calculated using a recent ECCC and HC ADI (ECCC and HC, 2016), methodology as described previously (Krishnan et al., 2010b), and a UF of 300 applied to the POD.

[i] A new BE was calculated using a recent US EPA RfD (US EPA, 2010), methodology as described previously (Hays and Aylward, 2008) and a UF of 10 applied to the POD.

inorganic arsenic was calculated as the sum of inorganic-arsenic exposure related urinary metabolites, viz. arsenate, arsenite, dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA).

Statistical estimates (GMs and percentiles of population concentration) for which the CV was between 16.6% and 33.3% are considered to have high sampling variability and caution is recommended when using these data (Health Canada, 2017c). To indicate that these data should be used with caution, these estimates are flagged in data tables, and data points based upon these estimates are marked with the plus (+) symbol in figures. When the CV for an estimate is greater than 33.3%, the value is considered unreliable and is therefore not interpreted using the biomonitoring screening values. GMs were not calculated when greater than 40% of measurements were below the limit of detection (LOD) and, in these cases, the assessment is based solely on the P95. In the calculation of statistical estimates, values below LOD were assigned a value of LOD/2. The estimates were calculated using the Statistical Analysis System (SAS) software and SUDAAN® statistical software package.

2.2. Selection and updating of biomonitoring screening values

The general methods for deriving BE and HBM-I values have previously been described (Hays et al., 2008a; Apel et al., 2017), and chemical specific derivations of various biomonitoring screening values are as reported in publications referred to in Tables 1–4.

2.2.1. Biomonitoring equivalents

In our analysis, BE values were preferred to other biomonitoring screening values when available. For the underlying exposure guidance values, preference was given to those from Health Canada, followed by those from the US EPA. However, in some cases, the exposure guidance value was chosen based on the most relevant route of exposure. Blood screening values derived using the BE approach are available for volatile organic compounds (VOCs) such as toluene and ethylbenzene. These blood screening values, derived in absence of specific PBPK model for several VOCs, are defined as the estimate of chemical specific steady-state blood concentrations associated with chronic oral and inhalation exposure at the corresponding US EPA RfD or Health Canada TDI concentrations (Aylward et al., 2010). Within this study, they are referred to as BE values. BE values based upon risk specific doses (RSD) from cancer risk assessments (i.e. BE_{RSD}) are available for acrylamide, arsenic and benzene. Since the St-Amand et al. (2014) study, an updated BE was published for 2,4-dichlorophenoxyacetic acid. In addition, BE values were recalculated for chemicals where the underlying exposure guidance values (e.g. RfD, TDI/ADI) have been revised since the original screening value publication (see details in Tables 1–4). These chemicals include molybdenum, cyfluthrin, deltamethrin, triclosan, GAVal, toluene, xylene and ethylbenzene.

2.2.2. Other biomonitoring screening values

HBM-I values were used for thallium and for the perfluoroalkyl substances, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) (Apel et al., 2017; Steckling et al., 2018). In the assessment of chlorpyrifos, the BGVs developed by Arnold et al. (2015) were used. These values represent the levels of urinary biomarker (3,5,6-trichloro-2-pyridinol, TCPy) for chlorpyrifos at which 10% of red blood cell cholinesterase inhibition is predicted to occur in 95% of a population following acute oral exposure.

2.3. Calculation of hazard quotients

For non-cancer endpoints, hazard quotients (HQ) were calculated as the ratio of the biomarker concentration, at the GM or P95, to the chemical-specific biomonitoring screening value:

$$HQ = [\text{biomarker}] \,/\, \text{biomonitoring screening value} \quad (1)$$

Table 2
Persistent environmental chemicals: biomarkers, exposure guidance values, corresponding biomonitoring screening values and biomonitoring data from the Canadian Health Measures Survey.

Chemical group	Chemical	Exposure guidance values (type, reference)	Biomonitoring screening value, value, reference	Unit and matrix	CHMS data				
					Cycle (years)	Age group, year	n	GM (95% CI)	P95 (95% CI)
Metals and trace elements	Cadmium	0.0005 mg/kg/d (RfD; US EPA, 1989)	BE: 1.7 Hays et al. (2008b)	μg/L whole blood	Cycle 4 (2014–2015)	3-5 (NS)	440	0.082 (< LOD–0.091)	0.2 (0.15–0.25)
						6-11 (NS)	909	0.095 (0.086–0.10)	0.19 (0.17–0.22)
						12-19 (NS)	863	0.12 (0.12–0.13)	0.29 (0.26–0.31)
						20-39 (NS)	775	0.18 (0.16–0.21)	0.52 (0.45–0.59)
						40-59 (NS)	809	0.25 (0.23–0.28)	0.62 (0.52–0.71)
						60-79 (NS)	840	0.34 (0.32–0.35)	0.88 (0.78–0.98)
						12-19 (S)	80	0.59[a] (0.4–0.88)	2.8 (2.0–3.7)
						20-39 (S)	270	1.8 (1.4–2.4)	5.2 (4.1–6.4)
						40-59 (S)	217	1.8 (1.4–2.3)	5.4[a] (2.9–7.8)
						60-79 (S)	136	2 (1.6–2.4)	5 (3.6–6.3)
Perfluoroalkyl substances	Perfluorooctane sulfonate (PFOS)	POD: 1–15 ng/ml	HBM-I: 5 Apel et al. (2017)	μg/L in plasma	Cycle 2 (2009–2011)	12–19	507	4.6 (4.0–5.2)	11 (9.2–13)
						20–39	362	6.2 (5.4–7.1)	19[a] (9.6–29)
						40–59	334	6.4 (5.7–7.2)	16 (13–19)
						60–79	321	9.4 (8.3–11)	21[a] (7.5–35)
	Perfluorooctanoic acid (PFOA)	POD: 1–10 ng/ml	HBM-I: 2 Apel et al. (2017)	μg/L in plasma	Cycle 2 (2009–2011)	12–19	507	2.1 (1.9–2.3)	4.1 (3.6–4.5)
						20–39	362	2.2 (1.9–2.5)	5.8 (3.9–7.6)
						40–59	334	2.2 (2.0–2.4)	4.4 (3.9–5)
						60–79	321	2.8 (2.4–3.2)	6.4 (4.6–8.1)

Abbreviations: BE: Biomonitoring equivalent, CHMS: Canadian Health Measures Survey, CI: Confidence interval, GM: Geometric mean, HBM-I: Human biomonitoring value I, NS: Non-smokers, P95: 95th percentile, PFOA: Perfluorooctanoic acid, PFOS: Perfluorooctanesulfonic acid, RfD: Reference dose, S: Smokers.

[a] Data should be used with caution as the coefficient of variation is between 16.6% and 33.3%.

Table 3
Volatile organic compounds: biomarkers, exposure guidance values, corresponding biomonitoring screening values and biomonitoring data from the Canadian Health Measures Survey.

Chemical	Exposure guidance values (type, reference)	BE value, reference	Unit and matrix	CHMS data				
				Cycle (years)	age group, year	n	GM (95% CI)	P95 (95% CI)
Bromoform	0.03 mg/kg/d (RfD; US EPA, 2005)	0.13 Aylward et al. (2008)	μg/L whole blood	Cycle 3 (2012–2013)	12–79	2496	NA[a]	0.01[b] (< LOD–0.015)
Chloroform	0.01 mg/kg/d (RfD; US EPA, 2001)	0.23 Aylward et al. (2008)	μg/L whole blood	Cycle 4 (2014–2015)	12–79	2527	NA[a]	0.043[b] (0.022–0.064)
Benzene	30 μg/m^3 (RfC, US EPA, 2003)	0.15 Hays et al. (2012)	μg/L whole blood	Cycle 4 (2014–2015)	12–79 (NS) 12–79 (S)	1896 400	0.025[b] (0.017–0.037) 0.14 (0.12–0.15)	0.097 (0.078–0.12) 0.42 (0.34–0.50)
Toluene	2.3 mg/m^3 (Indoor Air Guideline; Health Canada, 2011)	7.16[c] Aylward et al. (2010)	μg/L whole blood	Cycle 4 (2014–2015)	12–79 (NS) 12–79 (S)	1923 403	0.098 (0.072–0.13) 0.34 (0.27–0.41)	0.33[b] (0.19–0.48) 0.9 (0.73–1.1)
Ethylbenzene	0.022 mg/kg/d (TDI, Health Canada, 2014)	0.45[d] Aylward et al. (2010)	μg/L whole blood	Cycle 4 (2014–2015)	12–79 (NS) 12–79 (S)	2034 412	0.022 (0.018–0.026) 0.061 (0.052–0.072)	0.068 (0.04–0.088) 0.13 (0.11–0.16)
Xylenes	0.18 mg/m^3 (TC, Health Canada, 1996)	0.5[e] Aylward et al. (2010) See note	μg/L whole blood	Cycle 4 (2014–2015)	12–79 (NS) 12–79 (S)	1967 402	0.066 (0.054–0.080) 0.17 (0.15–0.19)	0.26 (0.17–0.34) 0.44 (0.32–0.55)
Styrene	1 mg/m^3 (RfC, US EPA, 1992)	3 Aylward et al. (2010)	μg/L whole blood	Cycle 4 (2014–2015)	12–79 (NS) 12–79 (S)	2050 417	0.048 (0.037–0.063) 0.093 (0.077–0.11)	0.1 (0.083–0.12) 0.19 (0.15–0.23)
Tetrachloroethylene	0.36 mg/m^3 (TC, Health Canada, 1996)	4 Aylward et al. (2010)	μg/L whole blood	Cycle 3 (2012–2013)	12–79	2453	NA[a]	0.17[b] (0.10–0.23)

Abbreviations: BE: Biomonitoring equivalent, CHMS: Canadian Health Measures Survey, CI: Confidence interval, GM: Geometric mean, NS: Non-smokers, P95: 95th percentile, RfC: Reference concentration, RfD: Reference dose, S: Smokers, TC: Tolerable concentration, UF: Uncertain factor.

[a] If > 40% of samples were below the limit of detection, the GM was not calculated.

[b] Data should be used with caution as coefficient of variation is between 16.6% and 33.3%.

[c] A new BE was calculated using a recent Health Canada Air Guidance value (Health Canada, 2011), methodology as described previously (Aylward et al., 2010) and applying an UF of 10 to the POD.

[d] A new BE was calculated using a recent Health Canada TDI (Health Canada, 2014), methodology as described previously (Aylward et al., 2010) and applying an UF of 25 to the POD.

[e] A new BE was calculated using a recent Health Canada TC (Health Canada, 1996), methodology as described previously (Aylward et al., 2010) and applying an UF of 1000 to the POD.

Table 4
Cancer reference value, corresponding Biomonitoring Equivalent at risk specific doses (BE_{RSD}) and biomonitoring data for substances measured in the Canadian Health Measures Survey.

Chemical group	Chemical (biomarker if different)	Cancer reference values (type, reference)	BE_{RSD} 1 × 10-4 risk level, reference	Unit and matrix	CHMS data						
					Cycle (years)	Age group, year n	5 th percentiles	25 th percentiles	50 th percentiles	75 th percentiles	95 th percentiles
Acrylamide	Acrylamide (GAVal)	0.5 $(mg/kg/d)^{-1}$ (Oral cancer slope factor, US EPA, 2010)	4.88[a] Hays and Aylward (2008)	pmol/g Hb whole blood	Cycle 4 (2014–2015)	3–79 (S) 265	39 (31–47)	64 (47–81)	97 (78–120)	150 (120–180)	250 (180–320)
						3–79 (NS) 2187	25[b] (< LOD–37)	40 (37–43)	51 (46–56)	68 (60–75)	110 (93–130)
Metals and trace elements	Arsenic (sum of iAs, MMA, DMA)	1.8 $(mg/kg/d)^{-1}$ (Oral cancer slope factor, Health Canada, 2006)	1.4[c] Hays et al. (2010)	µg As/L urine	Cycle 4 (2014–2015)	3–79 2567	1.9[b] (0.79–3.0)	3.3 (3.1–3.5)	4.8 (4.2–5.4)	8.4 (7.0–9.7)	20 (15–26)
VOCs	Benzene	2.2×10^{-6} - 7.8×10^{-6} $(µg/m^3)^{-1}$ (Inhalation Unit Risk, US EPA, 2000)	0.058–0.20[d] Hays et al. (2012)	µg/L whole blood	Cycle 4 (2014–2015)	12–79 (S) 400	0.029[b] (0.018–0.098)	0.098 (0.081–0.11)	0.14 (0.098–0.18)	0.21 (0.17–0.25)	0.42 (0.34–0.45)
						12–79 (NS) 1896	NA[e]	0.013[b] (< LOD-0.021)	0.03[b] (0.012–0.040)	0.048[b] (0.029–0.068)	0.097 (0.078–0.12)

Abbreviations: BE: Biomonitoring equivalent, BMDL: Benchmark Dose Lower bound; CHMS: Canadian Health Measures Survey, CI: Confidence interval, DMA: Dimethylarsinic acid, GAVal: Glycidamide hemoglobin valine terminal adducts, Hb: Hemoglobin, iAS: Inorganic arsenic, MMA: Monomethylarsonic acid, NS: Non-smokers, S: Smokers, VOCs: Volatile organic compounds.

[a] A new BE_{RSD} was calculated using a recent US EPA oral cancer slope factor (US EPA, 2010), methodology as described previously (Hays and Aylward, 2008). A more recent BMDL10 adopted by Health Canada (Health Canada, 2012) as the POD results in a BE_{RSD} of 4.39 pmol/g Hb, a value close to 4.88 pmol/g Hb used in this work, and does not modify risk interpretation of population percentiles.

[b] Data should be used with caution as coefficient of variation is between 16.6% and 33.3%.

[c] A more recent JECFA BMDL0.5 of 3 µg/kg/d (JECFA, 2011) is adopted by Health Canada in its Scientific Assessment in Support of a Lower Tolerance for Arsenic in Apple Juice. Using this BMDL results in a BE RSD of 1.45 µg As/L, a value close to 1.4µg As/L used in this work, and does not modify risk interpretation of population percentiles.

[d] HC (1996) TC05 = 15 mg/m^3. The risk-specific dose at a 1×10^{-4} risk level is 30 $µg/m^3$, within the range of US EPA for which the risk-specific dose at a 1×10^{-4} is 13–45 $µg/m^3$.

[e] Data is too unreliable to be published.

HQs near or exceeding a value of 1 suggest that exposure levels in the population are near or exceeding existing exposure guidance values.

BE values based upon risk specific doses at a risk level of 1×10^{-4} from cancer risk assessments (i.e. BE_{RSD}) were used in the calculation of cancer risk estimates corresponding to population exposures at the P5, P25, P50, P75 and P95 percentile using the equation below:

$$\text{Cancer risk} = ([\text{biomarker}] / BE_{RSD}) \times 10^{-4} \quad (2)$$

BE_{RSD} values provide an estimate of the steady-state concentrations in blood or urine that would result from chronic exposure, over a lifetime, at the risk specific doses. Linear extrapolation was assumed in the calculation of cancer risk estimates. Cancer risks exceeding 1×10^{-5} indicate that exposure levels may be exceeding the risk considered negligible by Health Canada (Health Canada, 2010a), and the substance involved should be considered as a priority for further evaluation.

Large variation in the blood or urinary concentrations of chemicals can be expected over the course of a day (intra-individual) or between individuals for highly transient chemicals such as VOCs and those described as non-persistent in this study (e.g. fluoride has a half-life of urinary elimination of approximately 6 h) (Aylward et al., 2012, 2015; Gurusankar et al., 2017). Consequently, for these chemicals, the tails of the concentration distribution (i.e. P5 and P95) may not be indicative of long-term exposure levels but rather transitory periods of low or high exposures. An evaluation of the central tendency of the population (GM) is more relevant for these chemicals as it allows more meaningful interpretation of population exposures (Aylward et al., 2013).

3. Results

Chemicals assessed using biomonitoring screening values based on non-cancer endpoints are shown in Table 1 (non-persistent chemicals), 2 (persistent chemicals) and 3 (volatile organic compounds). For each chemical, the tables present summary statistics from the CHMS for a relevant biomarker (parent chemical, metabolite or sum of metabolites), the exposure guidance value and the corresponding biomonitoring screening value, and references. Cancer reference values for known carcinogens measured as part of the CHMS (e.g. oral cancer slope factors, inhalation unit risk) along with population statistics relevant to the calculation of cancer risks are presented in Table 4.

3.1. Hazard quotients for non-cancer endpoints

3.1.1. Non-persistent chemicals

The HQ values at the GM and P95 concentrations for non-persistent chemicals in the Canadian population are presented in Fig. 1. For this exercise, a chemical was defined as non-persistent when its elimination half-life is relatively short (e.g., less than a day for some chemicals excreted in urine, or somewhat longer as in the case of the blood biomarkers of acrylamide (i.e. concentration of acrylamide hemoglobin adducts limited by the rate of red blood cell turnover)). Of the 14 non-persistent chemicals assessed, only four had HQ values greater than one at P95, namely fluoride, inorganic arsenic, 3-PBA and acrylamide. The HQs for fluoride in 3–5 and 6–11 year olds, calculated using the age-specific BE value (Aylward et al., 2015), slightly exceeded 1 at the P95 (1.23 and 1.07, respectively, for the two age groups) but not at the GM. The P95 estimate for 3–5 year olds has been flagged for high variability and, as such, caution is required when using this data. No exceedances were observed in the older age groups. For inorganic arsenic, the calculated HQ for 3–79 year olds at the GM was near one (0.797) while the HQ at the P95 exceeded one (3.13). For 3-PBA, a common metabolite of various pyrethroid pesticides, two screening-level BE values have previously been derived (Aylward et al., 2018). The tier 1 value of 1.7 μg/L in urine is highly conservative based on an assumption that all urinary 3-PBA is derived from an exposure to a pyrethroid pesticide with the most stringent exposure guidance value and the tier 2 value of 87 μg/L is less conservative but might be more realistic as it was derived based on a weighted relative exposure to different pyrethroid compounds (Aylward et al., 2018). For the tier 1 BE only, the HQ value exceeded 1 at the P95 (3.41, flagged for high variability) but not at the GM (0.25). Finally for acrylamide, which was assessed by comparing levels of two of its metabolites in blood, namely acrylamide haemoglobin adduct (AAVal) and glycidamide haemoglobin adduct (GAVal), HQs exceeded 1 for both biomarkers in smokers aged 3–79 years at the P95 (1.53 and 1.42, respectively for AAVal and GAVal). These exceedances were not seen in non-smokers for either biomarker.

3.1.2. Persistent chemicals

The HQ for persistent chemicals is shown in Fig. 2. In this analysis, HQs for cadmium at both the GM and P95 increased with age and were higher in smokers than non-smokers. Age-dependent increases in cadmium levels in the Canadian population have been noted previously for data from 2007 to 2009 and 2009–2011 of the CHMS (Garner and Levallois, 2016). For cadmium, non-smokers HQ values were below 1 while for smokers, HQ values exceeded 1 for all age groups at the GM (1.06–1.18) and P95 (1.65–3.18) with the exception of the GM for the youngest age group of 12–19 years included for these chemicals (0.35). The GM estimate for the aged group 12–19 years and the P95 estimate for the age groups 40–59 years are flagged for high variability and, as such, caution is required when using this data. For PFOA, HQs for all age groups were above 1 at the GM (1.05–1.40) and P95 (2.05–3.20). For PFOS, all HQs were above 1 at the GM (1.24–1.88), except the HQ for the youngest age group of 12–19 years (0.92). All HQs at the P95 (2.20–4.20) were above 1, with HQs for age groups 20–39 years and 60–79 years flagged for high variability.

3.1.3. Volatile organic compounds

The HQ values at the GM and P95 for VOCs in the Canadian population are presented in Fig. 3. All eight of the VOCs screened in this study had an HQ value below 1 at both the GM and P95 concentration with the exception of benzene in smokers. For this chemical, the HQ value at the GM for smokers approached 1 (0.93), and exceeded 1 at the P95 (2.80).

3.2. Cancer risks

The cancer risks for various percentiles of concentration in the Canadian general population (P5, P25, P50, P75 and P95) for biomarkers of acrylamide, inorganic arsenic and benzene are presented in Fig. 4. Most of the cancer risks calculated for these compounds were above the range of 10^{-5} to 10^{-6} considered to be essentially negligible risk (Health Canada, 2010a). Cancer risks calculated at different concentration percentiles for the acrylamide biomarker GAVal in non-smokers were close to 10^{-3} (ranging from 5.12×10^{-4} at P5, flagged with high variability, to 2.25×10^{-3} at P95). Cancer risks in smokers were higher (ranging from 7.99×10^{-4} at P5 to 5.12×10^{-3} at P95). For inorganic arsenic, cancer risk estimates were above the negligible risk range at all percentiles of the population assessed (ranging from 1.4×10^{-4} at P5, flagged for high variability to 1.4×10^{-3} at P95). Two BE_{RSD} were used for benzene corresponding to the lower bound and the upper bound of the cancer exposure guidance value range derived by the US EPA. For benzene in non-smokers, all of the cancer risks are above the range of negligible risks with the exception of the P25 (6.36×10^{-6}) when evaluated with the BE_{RSD} at upper bound which is the less conservative value. Caution is required when interpreting the cancer risks for benzene in non-smokers calculated at the P25, P50 and P75 due to high variability in the biomonitoring data. For benzene in smokers, cancer risks exceeded the negligible range when evaluated with the upper bound BE_{RSD} (ranging from 1.42×10^{-5} at P5 and 2.06×10^{-4} at P95) and with the lower bound BE_{RSD} (ranging from 4.97×10^{-5} at P5 to 7.20×10^{-4} at P95). Caution is required when interpreting the cancer risks for benzene in smokers calculated at the P5 due to high variability in the biomonitoring data.

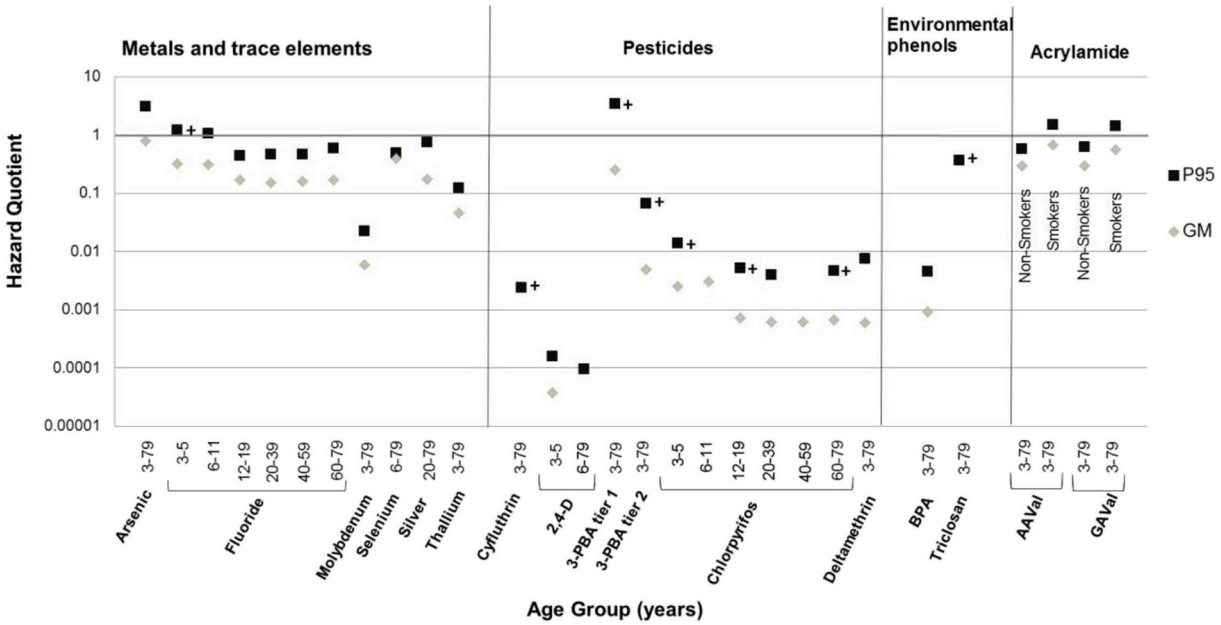

Fig. 1. Non-persistent environmental chemicals: Hazard quotients calculated with existing biomonitoring screening values and biomonitoring data from the Canadian Health Measures Survey for biomarkers of exposure for metals and trace elements (arsenic, fluoride, molybdenum, thallium), pesticides and environmental phenols in urine and for metals and trace elements (selenium, silver) and acrylamide in whole blood measured in the general Canadian population from 2009 to 2011 or 2014–2015. Abbreviations: +: HQ is to be used with caution as coefficient of variation is between 16.6% and 33.3%, 2,4-D: 2,4-Dichlorophenoxyacetic acid, 3-PBA: 3-Phenoxybenzoic acid, AAVal: Acrylamide hemoglobin valine terminal adducts, BPA: Bisphenol A, GAVal: Glycidamide hemoglobin valine terminal adducts, GM: Geometric mean, P95: 95th Percentile.

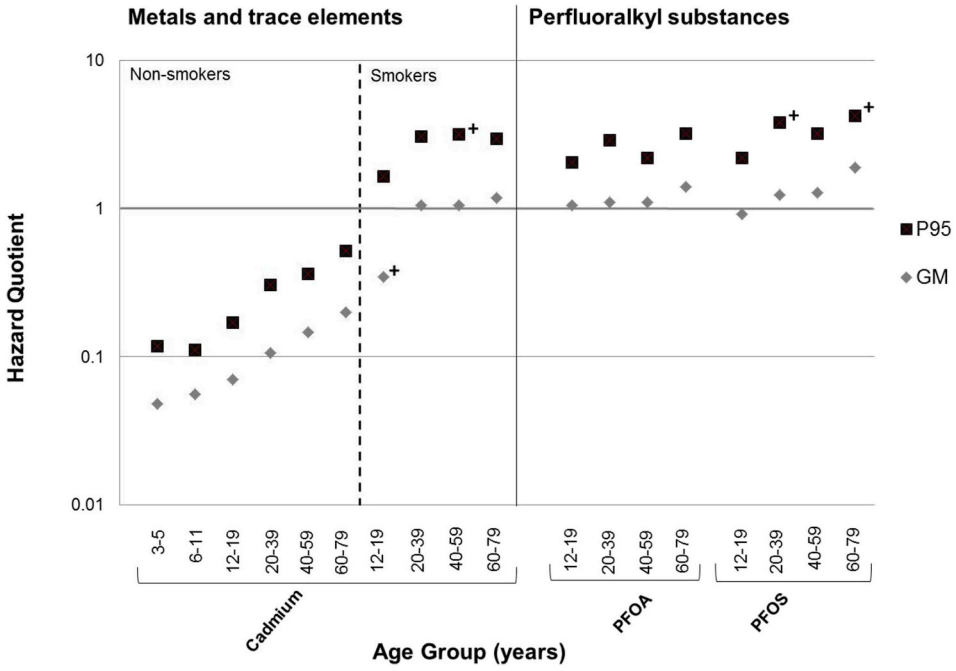

Fig. 2. Persistent environmental chemicals: Hazard quotients calculated with existing biomonitoring screening values and biomonitoring data from the Canadian Health Measures Survey for biomoarkers of exposure for cadmium in whole blood and perfluoroalkyl substances in plasma measured in the general Canadian population from 2009 to 2011 or 2014–2015. Abbreviations: +: HQ is to be used with caution as coefficient of variation is between 16.6% and 33.3%, GM: Geometric mean, PFOA: Perfluorooctanoic acid, PFOS: Perfluorooctane sulfonate, P95: 95th Percentile.

4. Discussion

4.1. Screening of biomonitoring data

This study analyzed CHMS biomonitoring data from various cycles in a health risk-based context building on the work of St-Amand et al. (2014). The current study continues the risk screening using more recent biomonitoring data and updated biomonitoring screening values.

For non-cancer endpoints, 17 of the 25 chemicals analyzed had HQs of less than 1 both at GM and P95 concentrations suggesting that exposure of the general population to these chemicals is occurring at levels below the current exposure guidance values. Specifically, the GM (or P95 when no GM is available) concentrations for eleven of these chemicals were 10–100 000 times lower than their respective BE values (HQs between 0.1 and 0.00001 for molybdenum, thallium, cyfluthrin, deltamethrin, chlorpyrifos, 2,4-D, BPA, bromoform, toluene, styrene, and tetrachloroethylene) and the HQs at the GM (or P95 when the GM is not available) for the other six chemicals fell between 1 and 0.1 (selenium, silver, triclosan, chloroform, xylenes and ethylbenzene). HQs exceeded 1 at the GM and/or P95 concentrations for eight

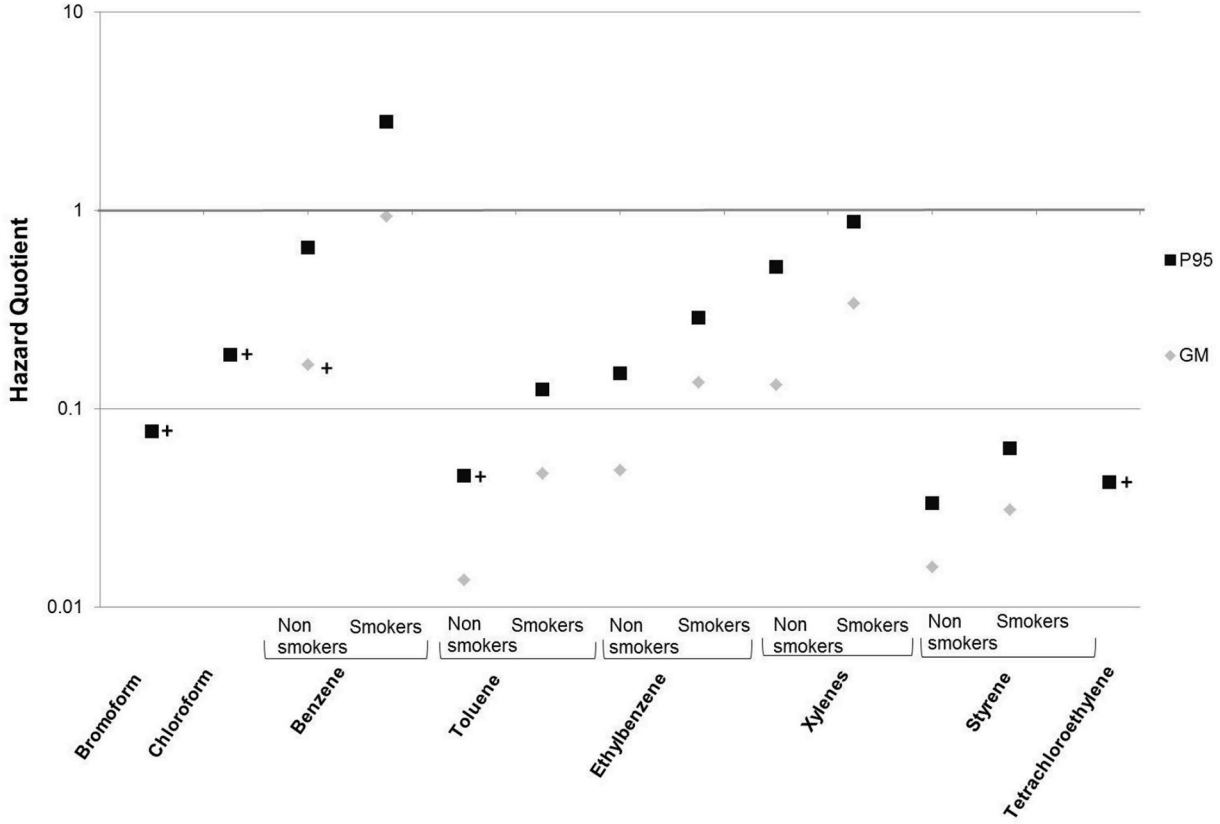

Fig. 3. Volatile organic compounds (VOCs): Hazard quotients calculated with existing biomonitoring equivalents and biomonitoring data from the Canadian Health Measures Survey biomarkers of exposure for VOCs in whole blood measured in the general Canadian population aged 12–79 years from 2012 to 2013 or 2014–2015. Abbreviations: +: HQ is to be used with caution as coefficient of variation is between 16.6% and 33.3%, GM: Geometric mean, P95: 95th Percentile.

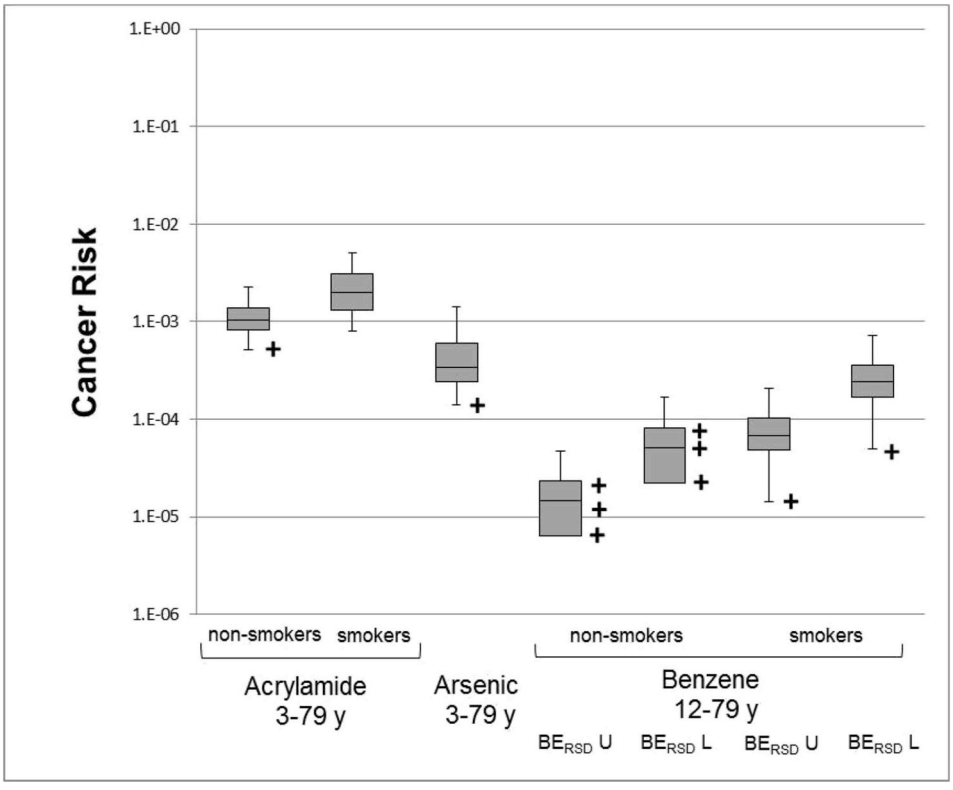

Fig. 4. Cancer risk for biomarkers of exposure for benzene and acrylamide (GAVal) in whole blood and urinary inorganic arsenic (sum of iAs, MMA and DMA) from the Canadian Health Measures Survey based on cancer exposure guidance values from Health Canada and U.S. EPA. BE_{RSD} used for benzene correspond to the lower bound (BE_{RSD} L) and the upper bound (BE_{RSD} U) of the cancer exposure guidance value range derived by the U.S. EPA. Medians are represented by the horizontal lines; boxes extend to the 25th and 75th percentiles, and whiskers extend to the 5th and 95th percentiles. Abbreviations: +: HQ is to be used with caution as coefficient of variation is between 16.6% and 33.3%, BE_{RSD}: Biomonitoring equivalent at risk specific dose, BE_{RSD} L: BE_{RSD} at lower bound, BE_{RSD} U: BE_{RSD} at upper bound, DMA: Dimethylarsinic acid, iAs: inorganic arsenic, MMA: Monomethylarsonic acid.

chemicals, namely fluoride, inorganic arsenic, 3-PBA, PFOA, and PFOS in the general population and acrylamide, cadmium, and benzene in smokers. These results indicate that exposure to these substances in the general and/or smoking Canadian population could be exceeding their respective exposure guidance values. In this study, cancer risks for benzene, acrylamide and inorganic arsenic in the general population fell above the range defined as essentially negligible (10^{-5}-10^{-6}) (Health Canada, 2010a).

BE values are applied with an assumption of chronic exposure, however for biomarkers with short half-life such as fluoride, large variations in concentrations can be expected within an individual over the course of a day. Consequently, high exposures represented by the P95 are not necessarily indicative of continuous exposure for non-persistent chemicals, particularly when measured in spot urine samples. Rather, for non-persistent chemicals, the upper bound of exposure may be more reflective of a transient peak of exposure and the central tendency (GM or P50) is more informative of long term exposure (Aylward et al., 2012; Gurusankar et al., 2017). In this study, this is relevant for fluoride, inorganic arsenic, 3-PBA and benzene for which biomarkers are rapidly eliminated and HQs at the GM are under 1. However, as the size of the sample in this analysis is large, it may be interesting to explore whether the P95 may actually be reflective of high level exposures to chemicals with short-elimination half-lives. Indeed, an examination of the CHMS biomonitoring data for short-lived chemicals demonstrates consistency of estimates across cycles at the tail of the population exposure, suggesting potential validity of use of the upper percentile estimates for short-lived chemicals in large population studies. For example, P95 concentrations of urinary BPA in cycles 1, 2, 3 and 4 are 6.9 μg/L, 6.7 μg/L, 6.6 μg/L and 6.0 μg/L, respectively. For substances with biomarkers having a longer half-life such as acrylamide, cadmium and perfluoroalkyl substances (PFOA and PFOS) both HQs at the GM and P95 are informative of population exposure because their blood or urine levels are more likely to remain constant over the course of days or weeks.

Results from the 2014–2015 data are consistent with the previous assessment of the same chemicals using data from 2007 to 2009 and 2009–2011. For example, St-Amand et al. (2014) identified exceedances of cadmium and inorganic arsenic concentrations over their respective biomonitoring screening values. The current results can also be compared with other national biomonitoring data screening studies such as those conducted on data from the U.S. National Health and Nutrition Examination Survey (NHANES). An interpretation of NHANES data from 2013 using BE values resulted in similar findings to those of St-Amand et al. (2014) and the present work. For example, HQs for inorganic arsenic exceeded 1 at the P95 concentration for the U.S. general population. HQs for acrylamide, cadmium and benzene also exceeded 1 at the P95 for smokers based on NHANES. Similar to our findings on cancer risks based on Canadian data, cancer risks in the U.S. population exceeded the 1×10^{-5}cancer risk level for benzene and acrylamide in both smokers and non-smokers, and inorganic arsenic in the general population (Aylward et al., 2013).

For the chemicals trichloroethylene, bromodichloromethane and dibromochloromethane measured in 2014–2015 of the CHMS, GM and P95 calculations were not possible due to low detection (i.e., more than 95% of measurements were below the LOD). Therefore, while BE values are available for these chemicals, it was not possible to calculate HQ values. Nevertheless, BE values for these chemicals are higher than their respective LODs. The low detection of these chemicals combined with the LODs being lower than the respective BE values indicates that exposures in the general population are below the current level of concern.

Finally, HQs greater than 1 at the GM or P95 for acrylamide, benzene, cadmium, fluoride, inorganic arsenic, 3-PBA, PFOA and PFOS, as well as cancer risks exceeding the range defined as essentially negligible (10^{-5}-10^{-6}) for benzene, acrylamide and inorganic arsenic suggest that these chemicals should remain as priorities for continued biomonitoring. For these chemicals, this screening exercise also provides evidence to support the findings of past risk assessments resulting in a number of risk management and mitigation measures implemented by Health Canada. Risk assessments under the Canadian Environmental Protection Act, 1999 (CEPA, 1999) have resulted in the listing of acrylamide, benzene, cadmium (inorganic), fluoride, inorganic arsenic, PFOA and PFOS on Schedule 1, List of Toxic Substances (Canada, 1999; Canada, 2018). The Act allows the federal government to control the importation, manufacture, distribution, and use of these chemicals in Canada. Accordingly, Health Canada has carried out a number of recent activities for these substances to reduce population exposure including proposed updates to the maximum levels of arsenic in apple juice and water in sealed containers, new guidelines for PFOS and PFOA in drinking water, and a health risk assessment of dietary cadmium (ECCC, 2016; Health Canada, 1996, 2017a, 2017b, 2017e, 2017d, 2018a, 2018b, 2018c). In addition, acrylamide, cadmium and inorganic arsenic have also recently been identified for further scoping to find new potential sources of exposure to assess and manage on the basis of previous screening activities with CHMS data including those by St-Amand et al. (2014) (ECCC and HC, 2019). Under the Pest Control Products Act (PCPA) (Canada, 2006b), various pyrethroid pesticides, for which 3-PBA is a metabolite, have been evaluated including re-evaluations of lambda-cyhalothrin and cypermethrin (Health Canada, 2017f, 2018d). A number of these mitigation measures have been implemented since the release of latest cycle of CHMS data (2014–2015) that have been used in the current analyses. These measures along with future studies and assessments and the ongoing collection of human biomonitoring data as part of the CHMS will help contribute to a better understanding of the potential health risks posed by these chemicals in the Canadian population.

4.2. Limitations of BE values and alternate screening approaches

Some limitations of this study are inherent to the use of BE values and other biomonitoring screening values. A number of these limitations, including the interim approach behind the derivation of these values, their population rather than individual purpose, as well as the lack of tools for the assessment of multi-pollutant exposures, are described elsewhere (Aylward et al., 2013; Kirman et al., 2012; St-Amand et al., 2014). An important limitation to this study is the absence of biomonitoring screening values for a number of chemicals measured in the CHMS including chlorophenols and neonicotinoid pesticides. This underpins the fact that our ability to monitor chemicals in humans exceeds our ability to interpret these data in a health risk context and that other ways to interpret risks posed by chemicals are required. Screening values can be challenging to derive due to limited experimental data or pharmacological models and the lack of a clear understanding of the mode of action for many environmental chemicals. Nevertheless, a number of BE values are currently being developed by Health Canada including for parabens and malathion.

Another limitation which has to be considered when interpreting the results is the fact that confidence in BE values can vary from one chemical to another. This confidence is based on biomarker specificity or relation of this biomarker to dose metrics associated with the endpoints of interest and the robustness of pharmacokinetics models. For example, confidence based on biomarker specificity is high for the AAVal and GAVal BE values (Hays and Aylward, 2008). In contrast, the confidence is only low to medium for the cyfluthrin BE as the biomarker is not directly related to the mode of action of this substance (Hays et al., 2009). Low biomarker specificity can lead to overestimation of the risks. For example, caution is required when interpreting data for biomarkers of inorganic arsenic and pyrethroid pesticides. In the case of inorganic arsenic, the concentration of DMA, the most detected urinary arsenic metabolite, drives the sum of the concentrations of inorganic-derived arsenic species calculated for this analysis. However, levels of urinary DMA has also been associated with direct consumption of DMA

contained in foods such as rice and seafood, and DMA derived from the metabolism of arsenolipids and arsenosugars contained in seafood (Hays et al., 2010). Consequently, the exposure to inorganic-derived arsenic as calculated in this study might be overestimated. In the case of 3-PBA, exceedances of the BE value are only observed when using the tier 1 BE value, which is based on a conservative assumption that all urinary 3-PBA arises from exposure to the most potent pyrethroid compound. A tier 2 BE value for 3-PBA is available which takes into account the estimated proportional contributions of several pyrethroids to an exposure (Aylward et al., 2018). When the tier 2 value is used, the HQ values fall below 0.1 (Fig. 1). Confidence in the pharmacokinetic models used in the development of a BE can be robust as for molybdenum, given that a method based on data from a study using several different daily doses of molybdenum and conducted in a controlled metabolic research station is used to convert a daily dose of molybdenum to urinary excretion rate, or less robust as for deltamethrin for which a urinary BE is derived based on a dataset from a study using only a single oral dose (Aylward et al., 2011; Hays et al., 2016).

Not only are the biomarkers and pharmacokinetic models important for confidence in a BE, but also are the exposure guidance value that were used to derive the BE. These values and associated confidence can also differ greatly between regulatory agencies due to differences in studies used, points of departure and uncertainty factors. Consequently, each BE will be only as strong as the exposure guidance value on which it is based. For example, several BE values exist for benzene including the value of 0.15 µg/L used in this study based on a US EPA assessment and a less conservative value of 0.29 µg/L based on a California Reference Exposure Level. The major difference was the use of two different human studies with different endpoints of interest (Hays et al., 2012). Although BE values are used here only to identify chemicals that may require further evaluation, recent risk assessments have used biomonitoring screening values alongside biomonitoring data from the CHMS to evaluate risks to human health (Health Canada, 2018e; Zidek et al., 2017). Such uses exist for several chemicals including four assessed in this study namely selenium, thallium, molybdenum and silver (Health Canada, 2016). Therefore, the increasingly broad use of BE values demonstrates a need to continue to improve the accuracy and relevance of these values based on available exposure guidance values and pharmacokinetic data. More recently, Phillips et al. (2014) proposed a stochastically based Monte Carlo approach for calculating a distribution of BE values for a chemical taking into account the variability in physiology and pharmacokinetics at different exposure levels. A distribution of BE values rather than a single BE may be a more useful tool to more appropriately evaluate both the central tendency and the tail (e.g. 95th percentile) of a population distribution of biomonitoring data, especially for short-lived chemicals where higher biomonitoring concentrations may reflect elevated acute or chronic exposures or simply the timing of exposure with respect to sample collection.

Chemicals assessed here for cancer risk are classified by the International Agency for Research on Cancer (IARC) as group 1 (carcinogenic to humans, arsenic and benzene) or group 2A (probably carcinogenic to humans, acrylamide). Nevertheless, it is important to consider some uncertainties associated with cancer risk estimation using screening values derived based on cancer slope factors. Firstly, the slope factors or unit risks associated with lifetime exposure to a chemical may themselves vary between different agencies (e.g. US EPA, Health Canada). Further, the cancer slope factors or unit risks have been arrived at using linear models in conjunction with a low-dose extrapolation approach. Whereas this is an accepted approach for the estimation of cancer risks, it is plausible that the uncertainties associated with the shape of dose response or mode of action at low-doses may potentially affect results including possible overestimation or underestimation of the risks. Considering the uncertainty associated with low-dose cancer risk extrapolation, a margin of exposure approach that uses a point of departure such as the benchmark dose (BMD) associated with a low, measurable (e.g. 10% increase over background cancer incidence) response in an experimental or epidemiological study is gaining significance in the assessment of cancer risks for genotoxic, non-threshold carcinogens.

4.3. Future work

This type of analysis provides useful evidence for risk assessors and risk managers for further assessment and/or follow-up related to the assessment and management of chemical exposures. In this sense, continued screening of CHMS data when new biomonitoring screening values and/or new biomonitoring data are available is required. Ongoing revisions to environmental and dietary questionnaires in CHMS to better capture exposure sources will augment follow-up activities including re-evaluation and mitigation of exposures.

It would also be interesting to analyse the risk posed by combined exposure of chemicals with chemical-specific biomonitoring screening values. For example, the Hazard Index is an approach assuming dose addition in tissues and used in an assessment of VOC data from NHANES by Aylward et al. (2013) and Kirman et al. (2012). A similar exercise can be done with CHMS chemicals that have known interactions and shared end-points; and this could provide further information to support regulatory evaluation. Benzene is an example of a chemical for which this exercise may be of use as it has HQs above 1 and is known to interact with other organic volatile compounds such as toluene, xylene and ethylbenzene (Haddad et al., 1999).

5. Conclusion

This study provides a unique assessment of chemical exposures in a health risk based context at the population level in Canada using the most recent CHMS biomonitoring data. It is a rapid screening approach to identify environmental chemicals to which the general population may be exposed at levels near or exceeding existing risk assessment-based exposure guidance values. This presents an additional layer of exposure-based prioritization building upon the original prioritization process carried out when the chemicals were initially selected for inclusion in the CHMS. Consequently the identified chemicals can be seen as priorities for advanced examination including investigation of sources and pathways of exposure. This may subsequently lead to targeted actions to eliminate and mitigate exposure sources and reduce associated health risks. As many regulatory actions are underway or have already been implemented for these chemicals, this study also provides evidence to support these actions. The ongoing collection and screening of human biomonitoring data from the CHMS will help track exposures to these priority chemicals in the Canadian population and support the ongoing work to mitigate exposures and reduce health risks.

Conflicts of interest

None to declare.

Acknowledgement

The authors would like to acknowledge Claude Viau, Cheryl Khoury, Jeff Willey, Andy Nong, Michelle Gagné, Kristin Macey and Scott Blechinger for their review or/and valuable insights during preparation of this work. The Canadian Health Measures Survey biomonitoring component is funded by the Chemicals Managment Plan, a Government of Canada initiative.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2019.07.009.

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International Journal of Hygiene and Environmental Health 223 (2020) 80–92

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

Prenatal exposure to perfluoroalkyl substances and associations with symptoms of attention-deficit/hyperactivity disorder and cognitive functions in preschool children

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ARTICLE INFO

Keywords:
Perfluoroalkyl substances (PFASs)
Attention-deficit/hyperactivity disorder (ADHD)
Cognitive functions
Prenatal
The Norwegian Mother
Father and Child Cohort Study (MoBa)

ABSTRACT

Background: Perfluoroalkyl substances (PFASs) are persistent organic pollutants that are suspected to be neurodevelopmental toxicants, but epidemiological evidence on neurodevelopmental effects of PFAS exposure is inconsistent. We investigated the associations between prenatal exposure to PFASs and symptoms of attention-deficit/hyperactivity disorder (ADHD) and cognitive functioning (language skills, estimated IQ and working memory) in preschool children, as well as effect modification by child sex.

Material and methods: This study included 944 mother-child pairs enrolled in a longitudinal prospective study of ADHD symptoms (the ADHD Study), with participants recruited from The Norwegian Mother, Father and Child Cohort Study (MoBa). Boys and girls aged three and a half years, participated in extensive clinical assessments using well-validated tools; The Preschool Age Psychiatric Assessment interview, Child Development Inventory and Stanford-Binet (5th revision). Prenatal levels of 19 PFASs were measured in maternal blood at week 17 of gestation. Multivariable adjusted regression models were used to examine exposure-outcome associations with two principal components extracted from the seven detected PFASs. Based on these results, we performed regression analyses of individual PFASs categorized into quintiles.

Results: PFAS component 1 was mainly explained by perfluoroheptane sulfonate (PFHpS), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS) and perfluorooctanoic acid (PFOA). PFAS component 2 was mainly explained by perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA) and perfluorononanoic acid (PFNA). Regression models showed a negative association between PFAS component 1 and nonverbal working memory [β = -0.08 (CI: -0.12, -0.03)] and a positive association between PFAS component 2 and verbal working memory [β = 0.07 (CI: 0.01, 0.12)]. There were no associations with ADHD symptoms, language skills or IQ. For verbal working memory and PFAS component 2, we found evidence for effect modification by child sex, with associations only for boys. The results of quintile models with individual PFASs, showed the same pattern for working memory as the results in the component regression analyses. There were negative associations between nonverbal working memory and quintiles of PFOA, PFNA, PFHxS, PFHpS and PFOS and positive associations between verbal working memory and quintiles of PFOA, PFNA, PFDA and PFUnDA, with significant relationships mainly in the highest concentration groups.

Conclusions: Based on our results, we did not find consistent evidence to conclude that prenatal exposure to PFASs are associated with ADHD symptoms or cognitive dysfunctions in preschool children aged three and a half years, which is in line with the majority of studies in this area. Our results showed some associations between PFASs and working memory, particularly negative relationships with nonverbal working memory, but also

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https://doi.org/10.1016/j.ijheh.2019.10.003
Received 21 June 2019; Received in revised form 4 October 2019; Accepted 7 October 2019

positive relationships with verbal working memory. The relationships were weak, as well as both positive and negative, which suggest no clear association – and need for replication.

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders, affecting approximately 5% of children worldwide (Polanczyk et al., 2007). ADHD is characterized by inattention, impulsivity and hyperactivity (American Psychiatric Association, 2013). Symptoms of ADHD are often present in the preschool years (Skogan et al., 2014), which is also an important period for the development of cognitive functions and language (Garon et al., 2008; Rice et al., 2008). Childhood ADHD is 2–9 times more prevalent in boys, but there are smaller sex differences in population-based samples compared with clinical samples (Nussbaum, 2012; Polanczyk et al., 2007). The reasons for sex differences are not known, but it has been hypothesized that a higher degree of externalizing behavior problems among boys with ADHD compared to girls may result in a sex-based referral bias (Biederman, 2005; Martin et al., 2018; Nussbaum, 2012). The underlying causes of ADHD are most likely interplays between genetic and non-genetic factors (Faraone et al., 2005; Thapar et al., 2013). While the role of heritability in the etiology of ADHD is well documented (Chang et al., 2013; Faraone et al., 2005), knowledge about how environmental factors may affect the development of ADHD is still scarce (Thapar et al., 2013). Exposure to environmental toxicants during pregnancy has gained increased interest as a risk factor for neurodevelopmental disorders (Grandjean and Landrigan, 2014). During pregnancy, toxicants can be transferred from mother to fetus via the placenta (Grandjean and Landrigan, 2014; Gützkow et al., 2012; Kato et al., 2014). The fetus has an undeveloped blood-brain barrier and limited ability to eliminate toxicants (Grandjean and Landrigan, 2014) therefore, exposure to toxicants *in utero* may disrupt normal brain development and hence be a potential risk factor for impaired cognitive functions and neurodevelopmental disorders such as ADHD or related symptoms (Grandjean and Landrigan, 2006, 2014; Kajta and Wójtowicz, 2013).

Compared to other environmental toxicants, poly- and perfluoroalkyl substances (PFASs) are among those with highest levels in human blood, including pregnant women (Haug et al., 2018; Mariussen, 2012). PFAS is a large group of synthetic compounds developed for use in a multitude of different products (e.g. firefighting foam, textiles, cooking pans, and food packaging) because of its water, oil and dirt repelling properties (Buck et al., 2011; Kissa, 2001). Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are the two most prevalent and extensively studied PFASs. Due to phase-out by major producers as well as international legislation and reduced use, levels of some PFASs have declined in the environment during the last 10–15 years (EFSA CONTAM Panel, 2018; Mariussen, 2012). However, several PFASs are highly persistent in the environment and in humans, and PFOS and PFOA have estimated biological half-lives of around two to five years in the human body (EFSA CONTAM Panel, 2018; Lau et al., 2007). Furthermore, new types of PFASs with longer half-lives have replaced PFOS and PFOA (Sunderland et al., 2019; Wang et al., 2017). Bound to protein-rich tissues, many of the PFASs will accumulate in animals and magnify up the food chain (Conder et al., 2008; Houde et al., 2006). In Norway, the major sources of exposure to these substances are food, especially seafood (Haug et al., 2010). Experimental rodent studies suggest that PFASs may be developmental neurotoxicants (Grandjean and Landrigan, 2014; Johansson et al., 2009; Mariussen, 2012; Viberg et al., 2013). Importantly, PFASs have endocrine-disruptive abilities and can affect the maternal and fetal thyroid hormone systems, which are essential for a normal development of the fetal nervous system and brain (De Cock et al., 2012; Mariussen, 2012; Tran and Miyake, 2017). Experimental animal studies have suggested that there are sex differences regarding the elimination of PFASs and that it is possibly linked to prenatal gonadal hormone levels (Lau et al., 2007). In addition, studies suggest interaction between PFAS exposure and sex hormone homeostasis (Kjeldsen and Bonefeld-Jørgensen, 2013; Mariussen, 2012).

Results from epidemiologic studies investigating prenatal exposure to PFASs and neurodevelopment, such as ADHD diagnosis/symptoms and cognitive functions, are inconsistent (Liew et al., 2018a; Rappazzo et al., 2017). Most studies on ADHD or related symptoms report no associations (Fei and Olsen, 2011; Lien et al., 2016; Liew et al., 2015; Ode et al., 2014; Oulhote et al., 2016; Quaak et al., 2016; Stein et al., 2013; Strøm et al., 2014; Vuong et al., 2018). Two studies report positive associations between prenatal PFAS exposure and hyperactivity symptoms (Høyer et al., 2015, 2018). Research on prenatal exposure to PFASs and offspring cognitive functions report weak or lack of associations, or report conflicting evidence (Chen et al., 2013; Harris et al., 2018; Jeddy et al., 2017; Liew et al., 2018b; Stein et al., 2013; Vuong et al., 2019; Zhang et al., 2018). However, one study did report negative associations between higher PFAS levels and lower IQ in the child at ages five and eight (Wang et al., 2015). In addition, another study reported associations between higher prenatal PFOS levels and increased impairments in metacognition (Vuong et al., 2016). Taken together, there is considerable uncertainty about the effect of PFASs as far as these types of neurodevelopmental outcomes are concerned. Among the studies, there is a large variety of different instruments and methods and several of them have small sample sizes. Furthermore, no previous studies have investigated prenatal PFAS exposure in relation to ADHD symptoms using neuropsychological assessments of three-year-old children.

The present study's overall aim is to investigate the associations between prenatal exposure to PFASs and ADHD symptoms, language skills, estimated IQ and working memory in preschool children, as well as to investigate effect modification by child sex of these associations.

2. Methods

2.1. Study design and participants

2.1.1. The Norwegian Mother, Father and Child Cohort Study

The Norwegian Mother, Father and Child Cohort Study (MoBa) is an ongoing prospective population-based cohort study conducted by the Norwegian Institute of Public Health (Magnus et al., 2016). The cohort now includes over 114,000 children, 95,000 mothers, and 75,000 fathers. Participants (41% participation rate) were recruited from all over Norway from 1999 to 2008. Pregnant women were invited to participate when scheduling their first free ultrasound scanning around the 17th week of pregnancy. Blood samples were collected from both parents in pregnancy and from the mother and child at birth (Magnus et al., 2016).

2.1.2. The ADHD study

The current paper is based on the ADHD Study, a sub-study of children with high levels of ADHD symptoms. The children were identified through the MoBa questionnaire that mothers completed when the child was three years of age (Overgaard et al., 2018). This questionnaire included 11 items about ADHD, of which six items were from the Child Behavior Checklist/1.5–5 (Achenbach and Rescorla, 2010) and five items from the DSM-IV-TR criteria for ADHD (American Psychiatric Association, 2000). Children with scores ≥ 90th percentile

on these 11 items (n = 2798) were invited to participate in a clinical assessment, along with randomly selected children from the MoBa cohort (n = 654). Among those eligible for the present sub-study, 149 children with high scores on autistic symptoms were sampled to another MoBa sub-study of autism (Fig. 1). In total, about 35% agreed to participate in the present sub-study. From 2007 to 2011, 1195 children (mean age: 3.5 years, age range: 3.1–3.8 years) took part in a one-day clinical assessment including a neuropsychological assessment with the child and a diagnostic interview with one of the parents, usually the mother. Details about the screening criteria are described elsewhere (Overgaard et al., 2018). In the overall sample, the proportions of girls and boys who met symptom criteria for ADHD diagnosis according to the parent interview were about 17% and 20%, respectively (Overgaard et al., 2018, 2019).

When excluding non-singleton pregnancies, withdrawals from the study, and those without available blood samples, the total number of mother-child pairs was 944 in the present study (Fig. 1). None of the children participating in this study had been or was medicated for ADHD at the time of assessment. We used version 9 of the MoBa quality-assured data files. MoBa is regulated under the Health Registry Act. Participation in MoBa is based on written informed consent from the parents. The ADHD Study has approval from the Regional Committee for Health Research Ethics for Southeast Norway. Participation in the clinical assessments of the ADHD Study required an additional written informed consent. This study was approved by The Regional Committee for Medical Research Ethics (ref. nu. 2012/985–1).

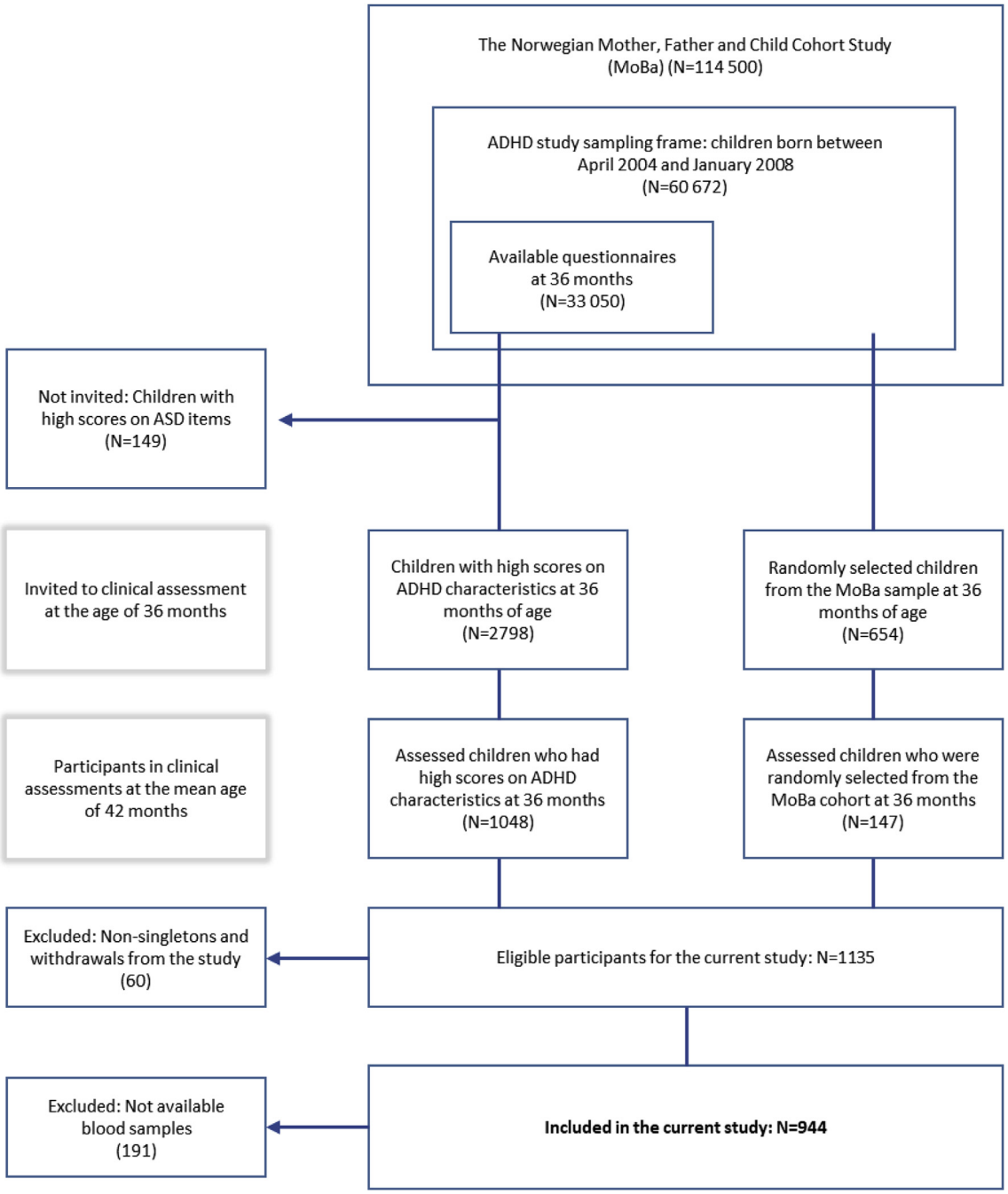


Fig. 1. Recruitment of participants and inclusion in the current study in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2004–2008. Abbreviations: Attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), The Norwegian Mother, Father and Child Cohort Study (MoBa).

2.2. Exposures

The present study used maternal plasma samples from week 17 of gestation to measure PFAS levels. Details about the sampling procedure and handling and storage in the MoBa biobank is described elsewhere (Paltiel et al., 2014). Nineteen PFASs were determined in maternal plasma (Table S1), using liquid chromatography-triple quadruple mass spectrometry (LC-MS/MS) as described previously (Haug et al., 2009). This method has been thoroughly validated and used for determination of more than 5000 serum/plasma samples so far, including approximately 2000 samples from MoBa (Singer et al., 2018). Only PFASs with levels above limit of quantification (LOQ) in > 80% of the plasma samples were included in the present study; PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS) and PFOS. Internal quality control samples and procedure blanks were analyzed along with each batch of samples to ensure high quality of the determinations throughout the project. The samples were also randomized to batch.

2.3. Outcomes

2.3.1. ADHD symptoms

Diagnostic assessments of the children were based on the Preschool Age Psychiatric Assessment (PAPA) interviews with their parents (Egger and Angold, 2004). The ADHD classification/diagnosis defined by PAPA is not equivalent to clinical ADHD diagnoses that would require a broader assessment, including multiple sources of information and informants. In the ADHD Study, only symptoms lasting ≥3 months were counted as present. Psychologists, psychiatrists, or trained graduate psychology students conducted the interviews. When graduate students conducted the interviews, they were under supervision by a child psychologist or a psychiatrist. As an inter-rater reliability check, a separate rater who was blind to the parent and teacher screen ratings, rescored audiotapes of 79 randomly selected assessment interviews. The average intra-class correlations (ICCs) were 0.97 for hyperactivity and impulsivity (HI) symptoms, 0.99 for inattention (IA) symptoms, and 0.98 for the total number of ADHD symptoms. In the present study, ADHD symptom sum scores were based on symptoms of inattention, hyperactivity, and impulsivity from the PAPA interview. Higher scores indicated more ADHD symptoms and higher severity.

2.3.2. Expressive language skills

Experienced clinicians with specialization in pediatric neuropsychological assessments conducted the tests of cognitive abilities of the children, including language skills, estimated IQ, and working memory. Expressive language skills were measured with Child Development Inventory (CDI). The CDI is a questionnaire for assessment of children from 15 months to six years of age, where teachers and parents fill in the questionnaires (Ireton and Glascoe, 1995). The questionnaire is consistent with results from psychometric tests of children and has good sensitivity and specificity (> 80%) of identifying delayed development in children (Doig et al., 1999). In the CDI, delayed language is defined as at least 1.25 standard deviations below the mean (Rohrer-Baumgartner et al., 2016). In the present study, we used the language subscale that was filled in by the preschool teacher. The subscale contains 50 items that assess primarily expressive communication, from simple gestures to complex language expressions. We used the daycare teacher report instead of parental report, as preschool teachers generally are assumed to have a good reference base for the evaluations (Rohrer-Baumgartner et al., 2016). A higher score indicated better language skills.

2.3.3. Estimated IQ (verbal and nonverbal)

Intelligence quotient (IQ) refers to performance on standardized tests measuring intellectual abilities (Rohrer-Baumgartner et al., 2014). Two subtests from Stanford Binet Intelligence scales (5th edition), were used to assess estimated IQ. This test battery has good psychometric properties and is standardized for ages two to 85 (Roid, 2003). In the present study, an estimated verbal IQ score was based on the "Vocabulary Task" where the child is requested to point at different body parts or name objects (toys) and explain the meaning of selected words. An estimated nonverbal IQ score was based on the "Object Matrices Task", that entails tasks such as detection of shapes that are alike and to fill in a missing shape on the basis of abstract reasoning. The verbal task is a measure of knowledge and the nonverbal task is a measure of fluid reasoning, which together is a good estimate of global ability (Roid, 2003). Both of these subtests have high loadings on the hierarchical g factor in cognitive ability batteries (Roid, 2003). The stop rule of discontinuing the test after four consecutive null scores was applied in all tests from this battery. A higher score indicated higher estimated IQ.

2.3.4. Working memory (verbal and nonverbal)

Working memory consists of a multicomponent cognitive system that allows for the rehearsal, storage and manipulation of information for a few seconds, and is a vital part of higher-order cognitive processes (Baddeley, 2012). Stanford Binet Intelligence scales (5th edition) was utilized to measure verbal and nonverbal working memory. Verbal working memory was assessed with the subtask "Memory for Sentences", where the child is asked to repeat sentences that increases gradually in length. Nonverbal working memory was measured with two subtasks; "Block Span" and "Delayed Response". In the Block Span test, the child is asked to tap blocks in the same order as the administrator. In the Delayed Response task, a small toy is placed under one of three cups when the child is watching; he or she is then asked to indicate where the toy is hidden after a short delay (Roid, 2003). A higher score indicated better working memory function.

2.4. Covariates

We obtained information on potential confounding variables from the Medical Birth Registry of Norway (MBRN) and MoBa questionnaires that were completed during pregnancy and up to child's age three years, as well as from questionnaires administered at three and a half years of age in the ADHD Study. Potential confounders were selected *a priori* based on existing literature and were guided by directed acyclic graphs (DAGs). Potential confounders included maternal age, maternal education, maternal fish intake, parity, maternal ADHD symptoms, child sex, premature birth, birth weight, maternal BMI, maternal smoking, maternal alcohol consumption, maternal anxiety/depression and maternal iodine intake. We did not include breastfeeding/breastfeeding duration because it temporally follows exposure, and therefore cannot confound prenatal PFAS concentrations. Based on the DAGs (Fig. S1 and Fig. S2), a minimal adjustment set (the minimal selection of variables to be adjusted for in order to avoid a biased result) was suggested to include maternal age, maternal education, maternal fish intake and parity using dagitty.net to estimate the total effect (Textor et al., 2011). We also included child sex in our final models as a confounder and effect measure modifier, because of the strong association between sex and the outcomes in question, and because effects of PFAS may be sexually dimorphic (Kjeldsen and Bonefeld-Jørgensen, 2013; Mariussen, 2012). When investigating ADHD symptoms and language skills as outcomes, the child's age at testing (in months) was also included as confounders, estimated IQ and working memory scores were already age-standardized. Maternal ADHD symptoms measured by the Adult ADHD Self-Report Scale (ASRS screener) (Kessler et al., 2007), was also included as a covariate in analyses of child ADHD symptoms as outcome.

2.5. Statistical analysis

Among the seven PFASs included in our study, four of them had

missing values due to levels below the LOQ. In addition, some of the covariates had missing values. To replace missing data, we ran multiple imputation by chained equations. In our analyses, we generated 50 datasets with the exposure and outcome variables, covariates and auxiliary variables (Rubin, 1976; Sterne et al., 2009) using the mi ice command in Stata (Royston, 2008). We used the method for interval-censored data and specified upper and lower limit for imputed results for PFASs as limit of detection (LOD) and zero, respectively (Royston, 2008). In the imputation model, we included the following (% missing): PFOA (0), PFNA (0.1), PFDA (17.5), PFUnDA (13.1), PFHxS (0), PFHpS (10.6), PFOS (0), child birth year (0), maternal age (0), maternal ADHD symptoms (1.0), maternal education (2.1), parity (0) maternal fish intake (1.6), child age at testing (0.5), child sex (0), maternal folate supplement (0), and the outcome variables. Some subjects were not included in the analyses due to missing values in an outcome variable (% missing): ADHD symptoms (0.1), estimated nonverbal IQ (1.0), estimated verbal IQ (0.8), nonverbal working memory (1.1), verbal working memory (18.6), and language (4.8). The pooling procedure used in the present article was mi estimate (Stata Press, 2017).

As a first step, we performed an exploratory principal component analysis (PCA) of log-transformed PFAS variables to investigate intercorrelation among the PFASs and to extract principal components. Oblimin rotation was chosen as this allows the components to be correlated, which can be the case when it comes to PFASs, independent of whether they are sulfonates or carboxylates. Delta was set at the default of zero. We performed multivariable analyses with negative binomial regression for ADHD symptoms and generalized linear regression analyses for language skills, nonverbal working memory, verbal working memory, estimated nonverbal IQ, and estimated verbal IQ with PFAS component scores as predictors, adjusting for the other component in the analyses. To optimize interpretation, the IQ and working memory scores were standardized into z-scores. For ADHD symptoms and language skills, sum scores were used. We also fitted models that included interaction terms of child sex and PFAS. In addition, we performed a sensitivity analysis in the models with PFASs as principal components, where we only included participants who were first-born. Based on significant findings from the component models, we further investigated the dose-response relationships between levels of individual PFASs categorized into quintiles and outcome variables in separate linear regression models, with the lowest quintile as the reference group. Investigation of dose-response relationships is important as this can give information on the function shape of PFAS-neurodevelopmental outcome relationships, which will not be interpretable by associations with component scores. We also performed a sensitivity analysis in the quintile models where fish intake was excluded as a covariate.

All regression models were expressed with regression coefficient (β) and accompanying 95% confidence intervals (CIs) or p-value (Wald's test, interaction term) with significance set at $p \leq 0.05$. The number of tests in this study was considerably reduced by using principal components as predictors in the regression models instead of single PFASs. Acknowledging that the number of tests performed is still fairly high ($n = 92$) and thus inflating the probability of type 1 error, we also evaluated the results with 99% CI and $p \leq 0.01$. This would correspond to Šidák correction to control for familywise error rate (false discoveries or type I errors) for $k = 92$ number of tests calculated by $100(1-\alpha)1/k$ % confidence intervals with $\alpha = 0.05$. Statistical analyses were performed in Stata version 15 (StataCorp, 2019).

3. Results

Characteristics of the study sample are displayed in Table 1. Mothers' mean age was 30.6 years. More than one third of the mothers had higher education (college or university) and almost all the mothers were married or cohabitating. The majority did not report smoking during pregnancy and most of them were primiparous. The sex distribution among the children was near equal with 51.4% boys. The sample characteristics by clinical symptoms are shown in the supplementary material (Table S2).

Table 2 shows the PFAS distribution of our sample including the mean, median and interquartile range of maternal PFAS concentrations during pregnancy. Three of the PFASs (PFOA, PFHxS and PFOS) were above LOQ in all measurements. These three also had the highest concentrations. The correlations among the PFASs are displayed in Table 3. The PFASs could largely be explained by two principal components and this model was chosen because it effectively captured the main correlation structure among the PFASs. Component one accounted for 42% of the covariation in the PFAS data with high loadings of PFOA, PFHxS, PFHpS and PFOS (Table S3). Component two accounted for 34% of the covariation and had high loadings of PFNA, PFDA and PFUnDA (Table S3). The distribution of the outcomes in the present study is presented in Table 4 and inter-correlations between the outcome variables are presented in Table 5.

The imputed and adjusted results are presented in this article, while complete case analyses (Table S4 and Fig. S3) and crude results (Table S5 and Fig. S4) are presented in supplementary material. The regression models showed a negative association between PFAS component one (mainly explained by PFOA, PFHxS, PFHpS and PFOS) and nonverbal working memory [β = -0.08 (95% CI: -0.12, -0.03)] (Table 6). This association remained with 99% confidence intervals. Between PFAS

Table 1
Characteristics of study population in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2004–2008.

Characteristic	Mean ± SD or n (%)
Total N	944
Maternal age at delivery (years)	30.58 ± 4.24
Missing (*n*)	0
Child sex	
Boy	485 (51.38)
Girl	459 (48.62)
Missing (*n*)	0
Maternal education	
< College/university	219 (23.70)
College/university	705 (76.30)
Missing (*n*)	20
Maternal marital status	
Married/Cohabitant	915 (96.93)
Single/Other	29 (3.07)
Missing (*n*)	0
Parity	
0	603 (63.88)
1 or more	341 (36.12)
Missing (*n*)	0
Maternal ADHD score	2.35 ± 0.62
Missing (*n*)	9
Maternal fish intake (g/day)	26.62 ± 17.73
Missing (*n*)	15
Child year of birth	
2004	109 (11.54)
2005	239 (25.32)
2006	303 (32.10)
2007–2008	293 (31.04)
Missing (*n*)	0
Smoking during pregnancy	
No	846 (89.62)
Yes	98 (10.38)
Missing (*n*)	0
Folate supplement	
No	166
Yes*	778
Missing (*n*)	0

Abbreviations: Attention-deficit/hyperactivity disorder (ADHD), standard deviation (SD). Note: *Any folate supplements between 4 weeks before and 8 weeks after conception.

Table 2
PFAS distribution in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

	N	% > LOQ	Mean	SD	Min	25%	50%	75%	Max
PFOA (ng/mL)	944	100%	2.61	1.18	0.33	1.77	2.50	3.21	9.81
PFNA (ng/mL)	943	99.89%	0.45	0.28	0.06	0.29	0.41	0.53	5.32
PFDA (ng/mL)	779	82.52%	0.19	0.14	0.05	0.10	0.15	0.23	1.77
PFUnDA (ng/mL)	820	86.86%	0.25	0.15	0.05	0.14	0.22	0.32	1.46
PFHxS (ng/mL)	944	100%	0.79	0.99	0.06	0.46	0.65	0.88	22.48
PFHpS (ng/mL)	844	89.41%	0.16	0.08	0.05	0.10	0.15	0.20	0.62
PFOS (ng/mL)	944	100%	12.32	5.38	2.38	8.77	11.51	14.84	42.23

Abbreviations: Perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorohexane sulfonate (PFHxS), perfluoroheptanesulfonic acid (PFHpS), perfluorooctane sulfonate (PFOS), limit of quantification (LOQ).

Table 3
Pearson correlations of PFASs in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

	PFOA	PFNA	PFDA	PFUnDA	PFHxS	PFHpS	PFOS
PFOA	1.00						
PFNA	0.67	1.00					
PFDA	0.50	0.77	1.00				
PFUnDA	0.26	0.57	0.71	1.00			
PFHxS	0.51	0.42	0.31	0.30	1.00		
PFHpS	0.64	0.47	0.39	0.32	0.61	1.00	
PFOS	0.62	0.51	0.44	0.42	0.54	0.80	1.00

Note: Correlation color coding goes from strong (green) to medium (yellow) and weak (red).

Table 4
Outcome distribution in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

	N	Mean	SD	Range
ADHD symptoms	943	5.54	6.10	0, 33
Language skills	899	49.31	7.02	15, 58
Nonverbal working memory	934	10.40	2.80	2, 17
Verbal working memory	768	7.60	1.91	5, 13
Nonverbal IQ	935	10.97	2.03	5, 18
Verbal IQ	936	9.72	2.11	2, 16

Note: Unscaled outcome variables. Abbreviation: Standard deviation (SD).

component two (mainly explained by PFNA, PFDA and PFUnDA) and verbal working memory there was a positive association [β = 0.07 (95% CI: 0.01, 0.12)] (Table 6). In the interaction models, we found effect modification by child sex (p = 0.01) for PFAS component two (PFNA, PFDA and PFUnDA) and verbal working memory. There was a stronger association in boys [β = 0.13 (95% CI: 0.06, 0.20)] compared to girls [β = 0.01 (95% CI: -0.06, 0.07)]. Among the girls, the confidence intervals contained the null (Table 6). The association for boys remained with 99% CI.

PFAS components were not associated with ADHD symptoms, language skills or estimated IQ (Tables 7 and 8). For ADHD symptoms, language skills, estimated IQ and nonverbal working memory, we observed no effect modification and no difference in the associations of PFAS component one and two by child sex (Tables 6–8).

The results from the sensitivity analysis, where we restricted our sample to first-born children, were fairly similar to the main analyses (Table S6). However, the association between component two (PFNA, PFDA and PFUnDA) and verbal working memory did not remain in the restricted models (Table S6).

We investigated dose-response relationships of significant relationships identified in the PFAS component models, using individual PFASs categorized into quintiles. For PFOA, PFHpS and PFOS there appeared to be a monotonic dose-dependent decrease in nonverbal working memory, however, only the fifth (highest) quintiles were significant; PFOA [β = -0.38 (95% CI: -0.61, -0.15)], PFHpS [β = -0.37 (95% CI: -0.58, -0.15)] and PFOS [β = -0.26 (95% CI: -0.47, -0.05)] (Fig. 2). A somewhat similar dose-response trend was observed for PFNA. In addition, there were negative associations between nonverbal working memory and the fourth quintile of PFHxS and the third quintile of PFNA (Fig. 2). The associations between PFOA and PFHpS with nonverbal working memory were also significant at 99% CI.

Quintile regression models with verbal working memory as outcome variable showed positive associations for the fifth quintiles of PFNA [β = 0.34 (95% CI: 0.10, 0.57)], PFDA [β = 0.32 (95% CI: 0.09, 0.55] and PFUnDA [β = 0.29 (95% CI: 0.05, 0.52)] (Fig. 3). In addition, there was a positive association between the third quintile of PFOA and verbal working memory (Fig. 3). With 99% CI, the associations with PFNA and PFDA remained. Excluding fish intake as a covariate in the sensitivity analyses did not change the association between PFASs and nonverbal and verbal working memory (Fig. S5).

Table 5
Pearson correlations of outcome variables in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

	ADHD symptoms	Language	Nonverbal IQ	Verbal IQ	Nonverbal WM	Verbal WM
ADHD symptoms	1.00					
Language	0.15	1.00				
Nonverbal IQ	-0.01	0.04	1.00			
Verbal IQ	0.09	0.23	0.10	1.00		
Nonverbal WM	0.15	0.12	0.02	0.22	1.00	
Verbal WM	0.06	0.16	0.02	0.26	0.13	1.00

Note: The variable ADHD symptoms is flipped. Correlation color coding goes from strong (green) to medium (yellow) and weak (red).

Table 6
Beta coefficients and 95% confidence intervals of adjusted regression models between PFAS components and working memory and interaction by child sex in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

PFAS components	Nonverbal working memory (n = 934)			Verbal working memory (n = 768)		
	All	Interaction term p = 0.863		All	Interaction term p = 0.105	
		Boys	Girls		Boys	Girls
Component 1: PFOA, PFHxS, PFHpS, PFOS	−0.08 (−0.12, −0.03)*	−0.08 (−0.14, −0.02)	−0.07 (−0.14, −0.01)	−0.01 (−0.06, 0.04)	0.02 (−0.04, 0.09)	−0.04 (−0.11, 0.02)
		Interaction term p = 0.662			Interaction term p = 0.012*	
Component 2: PFNA, PFDA, PFUnDA	0.03 (−0.02, 0.08)	0.02 (−0.05, 0.09)	0.04 (−0.03, 0.10)	0.07 (0.01, 0.12)	0.13 (0.06, 0.20)*	0.01 (−0.06, 0.08)

Note: A separate linear regression model (with multiple imputation) was conducted for each outcome with additional interaction analyses. The following PFASs most heavily loaded on component 1: PFHpS, PFOS, PFHxS and PFOA. The following PFASs most heavily loaded on component 2: PFDA, PFNA and PFUnDA. The PFASs were log transformed before computing principal components. Each regression model was adjusted for maternal education, age, parity, fish intake and child sex. Interaction term was tested with Wald's test. *Indicates significant results with 99% CIs.

4. Discussion

4.1. Main findings of the study

In the present study, we investigated the influence of prenatal exposure to seven PFASs (measured in maternal plasma in week 17 of pregnancy) on ADHD symptoms and cognitive functions in preschool children. With a sample of 944 mother-child pairs, this is one of the largest studies examining these exposure and outcome associations. In addition, few other studies have conducted neuropsychological tests of young preschool children in this particular research context. We accounted for the joint action of inter-correlated PFASs by extracting two principal components explaining 76% of the covariation in the PFAS data. Then we used component scores for component one and two as predictors in multivariable regression models with neurodevelopmental outcome variables. The results showed a negative association between component one (mainly explained by PFOA, PFHxS, PFHpS and PFOS) and nonverbal working memory. In quintile regression models, the individual PFASs of component one indicated a monotonic-like decrease in nonverbal working memory with increasing PFAS concentrations. Between PFAS component two (mainly explained by PFNA, PFDA and PFUnDA) and verbal working memory, there was a positive association. Quintile regression models with individual PFASs and working memory indicated some linear dose-response, but the relationships were both positive and negative, which complicates interpretation of these findings. There were no associations between PFAS components and ADHD symptoms, language skills or estimated IQ. Except for the weak negative associations between PFASs in component one, we did not find consistent evidence to suggest that prenatal exposure to PFASs is associated with ADHD symptoms or cognitive dysfunctions among preschool children.

4.2. Verbal and nonverbal working memory

In our study, we found some weak associations between PFAS components and working memory. Quintile models with individual PFAS concentrations and working memory were largely in agreement with the factor models. This also indicated that the use of PCA to reduce number of exposure variables was reasonable in this research context. However, we found results that were both positive (verbal working memory) and negative (nonverbal working memory), meaning that no clear pattern emerged. We found negative associations between nonverbal working memory and component one (PFOA, PFHxS, PFHpS and PFOS), where higher PFAS levels were associated with decreasing scores of nonverbal working memory. In the quintile models, the respective PFASs, in addition to PFNA, showed a similar pattern. Furthermore, there were indications of negative monotonic dose-response relationships for several of the PFASs (PFOS, PFOA and PFHpS), although only the fifth quintiles were significant. The associations between PFASs and nonverbal working memory appear to be somewhat novel, as few studies have investigated these particular exposure-outcome associations. Still, our results are partly in line with a study from the USA that analyzed exposure to prenatal PFASs and cognitive functions in children at the ages of five and eight years (Vuong et al., 2016).

Table 7
Beta coefficients and 95% confidence intervals of adjusted regression models between PFAS components and ADHD symptoms and language skills and interaction by child sex in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

PFAS components	ADHD symptoms (n = 943)			Language skills (n = 899)		
	All	Interaction term p = 0.212		All	Interaction term p = 0.078	
		Boys	Girls		Boys	Girls
Component 1: PFOA, PFHxS, PFHpS, PFOS	−0.01 (−0.07, 0.05)	−0.04 (−0.11, 0.03)	0.02 (−0.06, 0.09)	−0.09 (−0.42, 0.24)	0.12 (−0.29, 0.53)	−0.34 (−0.77, 0.09)
		Interaction term p = 0.526			Interaction term p = 0.024	
Component 2: PFNA, PFDA, PFUnDA	−0.00 (−0.06, 0.06)	−0.02 (−0.09, 0.06)	0.01 (−0.06, 0.09)	0.08 (−0.28, 0.43)	0.42 (−0.04, 0.89)	−0.24 (−0.69, 0.21)

Note: A separate regression model (with multiple imputation) was conducted for each outcome: negative binomial regression for ADHD symptoms and linear regression for language skills with additional interaction analyses. The following PFASs most heavily loaded on component 1: PFHpS, PFOS, PFHxS and PFOA. The following PFASs most heavily loaded on component 2: PFDA, PFNA and PFUnDA. The PFASs were log transformed before computing principal components. Each regression model was adjusted for maternal education, age, parity, fish intake, child sex and child age at testing. Child ADHD symptoms was adjusted for maternal ADHD symptoms. Interaction term was tested with Wald's test.

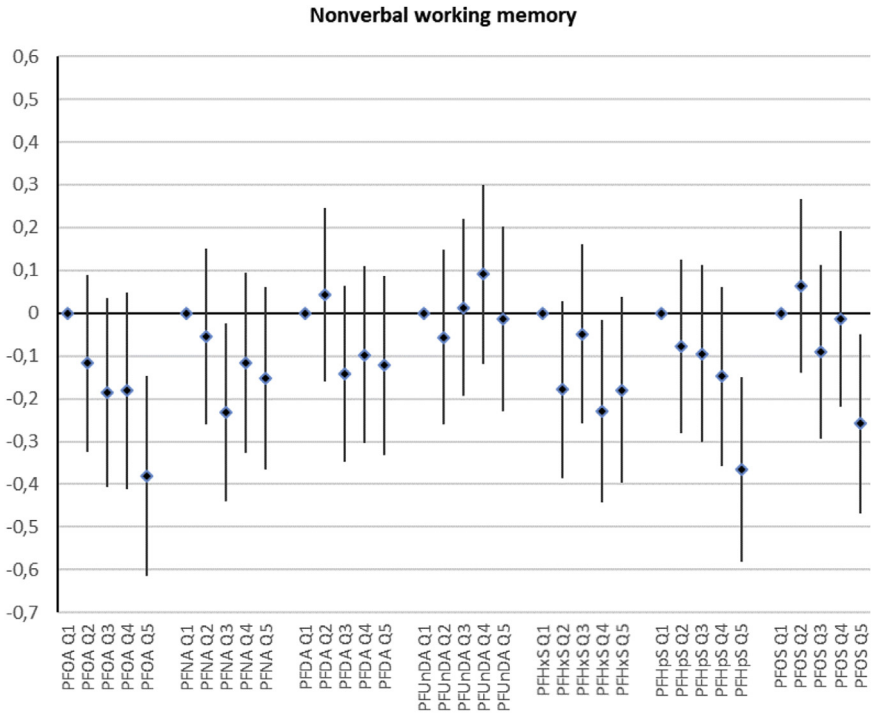
Table 8
Beta coefficients and 95% confidence intervals of adjusted regression models between PFAS components and estimated IQ and interaction by child sex in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

PFAS components	Nonverbal IQ (n = 935)			Verbal IQ (n = 936)		
	All	Interaction term p = 0.876		All	Interaction term p = 0.701	
		Boys	Girls		Boys	Girls
Component 1: PFOA, PFHxS, PFHpS, PFOS	0.00 (−0.05, 0.05)	0.00 (−0.05, 0.06)	−0.00 (−0.06, 0.06)	−0.02 (−0.07, 0.03)	−0.01 (−0.07, 0.04)	−0.03 (−0.09, 0.03)
		Interaction term p = 0.658			Interaction term p = 0.619	
Component 2: PFNA, PFDA, PFUnDA	−0.04 (−0.09, 0.01)	−0.03 (−0.10, 0.03)	−0.05 (−0.12, 0.01)	0.03 (−0.02, 0.08)	0.04 (−0.02, 0.11)	0.02 (−0.04, 0.09)

Note: A separate linear regression model (with multiple imputation) was conducted for each outcome with additional interaction analyses. The following PFASs most heavily loaded on component 1: PFHpS, PFOS, PFHxS and PFOA. The following PFASs most heavily loaded on component 2: PFDA, PFNA and PFUnDA. The PFASs were log transformed before computing principal components. Each regression model was adjusted for maternal education, age, parity, fish intake and child sex. Interaction term was tested with Wald's test.

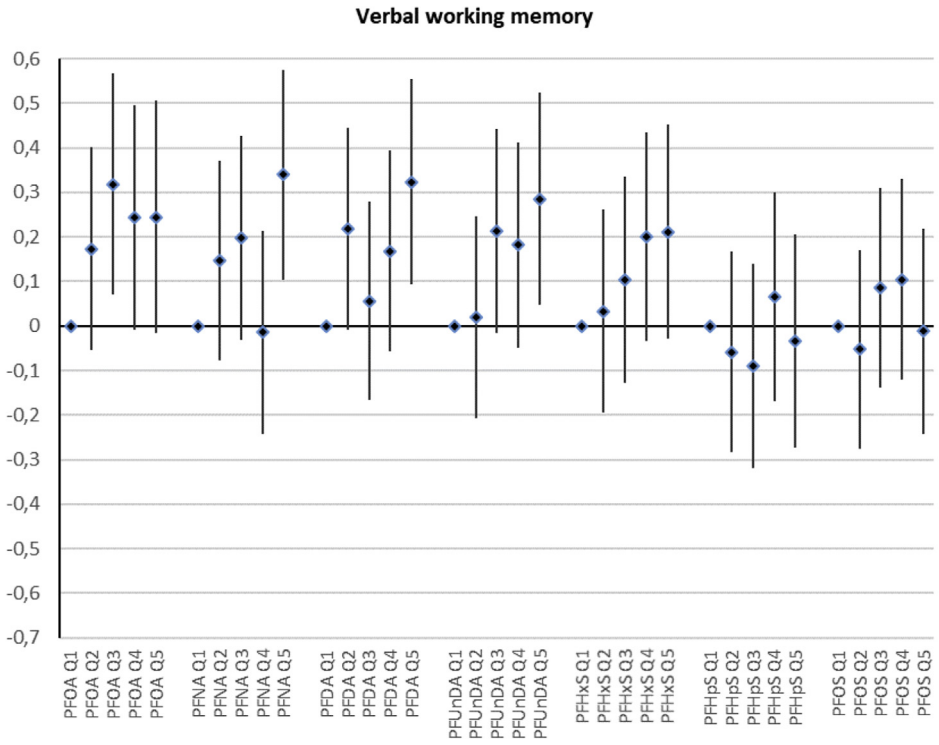
This study reported an association between increased levels of prenatal PFOS and impaired metacognition [β = 3.10 (95% CI: 0.62, 5.58)], which is dependent on multiple executive functions, such as working memory. However, there were no associations with the other investigated PFASs (PFOA, PFNA, PFHxS and PFDeA) and the sample size was quite small (n = 218) (Vuong et al., 2016).

Our study showed positive associations between verbal working memory and PFAS component two (PFNA, PFDA and PFUnDA) and the respective, individual PFAS quintiles, in addition to PFOA. Nevertheless, positive associations between PFASs and cognitive functions, such as language, IQ and memory have also been reported in other studies (e.g. Jeddy et al., 2017; Liew et al., 2018b; Stein et al., 2013; Vuong et al., 2019). Like our results, a recent study, reported positive associations between working memory and increases in prenatal levels of PFOA and PFNA (Vuong et al., 2019). That study used Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV) and assessed children at the age of 8 years (Vuong et al., 2019). A cohort study from the USA, investigating prenatal PFAS exposure and cognitive functions, reported both better and worse cognitive performance associated with prenatal PFAS exposure in three- and seven-year-olds (Harris et al., 2018). A possible mechanism behind the positive associations could be a result of PFASs that activate peroxisome



Note: Each PFAS by nonverbal working memory was modelled using a separate linear regression (with multiple imputation). The beta coefficient and 95% confidence intervals for each PFAS quintile are represented on the vertical axis (the reference level is the first quintile). Each regression model was adjusted for maternal education, age, parity, fish intake and child sex. Higher working memory score indicates better working memory function. Significant with 99% CIs: fifth quintiles of PFOA and PFHpS.

Fig. 2. Beta coefficients and 95% confidence intervals for regression models predicting nonverbal working memory (n = 934) from quintile categories of each PFAS in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.



Note: Each PFAS by verbal working memory was modelled using a separate linear regression (with multiple imputation). The beta coefficient and 95% confidence intervals for each PFAS quintile are represented on the vertical axis (the reference level is the first quintile). Each regression model was adjusted for maternal education, age, parity, fish intake and child sex. Higher working memory score indicates better working memory function. Significant with 99% CIs: fifth quintiles of PFNA and PFDA.

Fig. 3. Beta coefficients and 95% confidence intervals for regression models predicting verbal working memory (n = 768) from quintile categories of each PFAS in a nested study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort (MoBa), 2004–2008.

proliferator-activated receptors (PPARs) alpha and gamma, which have neuroprotective and central-nervous-system anti-inflammatory properties (Quaak et al., 2016; Stein et al., 2013). Research on working memory and prenatal PFAS exposure is scarce, and the results so far have been unclear and inconsistent. Our study showed some potential effects, especially for nonverbal working memory that needs to be replicated.

4.3. ADHD symptoms

In our study, we did not find any significant associations between PFAS exposure and ADHD symptoms, which is in line with other studies that have investigated ADHD symptoms as outcomes (Fei and Olsen, 2011; Quaak et al., 2016) as well as studies with ADHD diagnosis in children (Liew et al., 2015; Ode et al., 2014; Strøm et al., 2014). However, some studies have reported inverse relationships between prenatal PFAS exposure and ADHD symptoms or diagnosis (Lien et al., 2016; Liew et al., 2015; Stein et al., 2013; Vuong et al., 2018), although they concluded that there is a lack of evidence to support these associations. Contrary to our findings, birth cohort studies from Greenland, Ukraine and Poland found that increasing levels of PFOA in maternal blood during pregnancy was associated with increasing levels of hyperactivity in children between the ages of seven and nine [odds ratio = 3.1 (95% CI: 1.3, 7.2)] (Høyer et al., 2015). Furthermore, a recent study using data from these cohorts reported that increasing prenatal exposure to PFNA [odds ratio = 1.8 (95% CI: 1.0, 3.2) and PFDA [odds ratio = 1.7 (95% CI: 1.0, 3.1)] was associated with increasing hyperactivity symptoms in children between five and nine years in Greenland and Ukraine (Høyer et al., 2018). However, the authors did not rule out that it could be spurious findings (Høyer et al., 2018). Additionally, a cohort study from the Faroe Islands found significant positive associations between increasing levels of postnatal PFOA, PFNA, and PFDA and more hyperactivity/inattention problems among seven-year-olds, but not with prenatal PFAS exposure (Oulhote et al., 2016). Taken together, there remain uncertainties regarding the effect of prenatal PFAS exposure on ADHD symptoms and diagnosis, although most studies, like ours, have reported lack of associations.

4.4. Language skills

In accordance with our results, two other studies did not find associations between PFASs and language among two-year-olds and children between six and 12, respectively (Chen et al., 2013; Stein et al., 2013). Both of these studies had quite small sample sizes (n = 239 and n = 320 respectively). A larger study (n = 631 to 971), examining children at age three and seven and language comprehension, found only associations between one type of PFAS; 2-(N-methyl-perfluorooctane sulfonamido) acetate (MeFOSAA), but not the other seven PFASs (Harris et al., 2018). In that study, the second quartile of MeFOSAA was associated with higher receptive vocabulary scores (Harris et al., 2018). Other studies have reported positive relationships. A small study, examining prenatal PFAS exposure and reading ability among children at age five and eight, reported that increasing levels of PFOA, PFNA and PFOS were associated with improved reading skills at five years and at eight years of age (Zhang et al., 2018). Likewise, a study exploring early communication development among children at the ages of 15 and 38 months found both positive and negative associations between various prenatal PFASs and communication development among girls (Jeddy et al., 2017). The authors did point out that the results showed an inconsistent pattern of association across the

measured PFASs (Jeddy et al., 2017). Regarding language skills, there is limited knowledge about potential effects of prenatal PFAS exposure. In line with previous studies, we also report lacking associations.

4.5. Estimated verbal and nonverbal IQ

Our results showed no association between prenatal PFAS exposure and estimated IQ, neither verbal nor nonverbal. A large study (n = 1592) using data from the Danish National Birth Cohort found no associations between prenatal PFAS levels and IQ (full-scale, verbal and nonverbal) in their total sample, but some inconsistent associations in sex-stratified quartile analyses (Liew et al., 2018b). They concluded that overall, there was no evidence of an effect from prenatal PFAS on IQ in their study sample (Liew et al., 2018b). One study found associations between higher PFUnDA and lower nonverbal IQ at age five and higher PFNA levels with lower verbal IQ at age eight (Wang et al., 2015), however, the sample size was quite small (n = 120). In contrast, some studies have found positive associations between PFAS exposure and IQ measures (e.g. Harris et al., 2018; Stein et al., 2013; Vuong et al., 2019). A study that assessed children between six and 12 years of age reported that elevated PFOA levels was associated with improved full-scale IQ score (Stein et al., 2013). The study had a fairly small sample size (n = 320) and the authors concluded that the positive associations were imprecise and inconsistent (Stein et al., 2013). Likewise, a larger study (n = 631 to 971) found that higher prenatal levels of PFOS were associated with better nonverbal IQ among seven-year-olds (Harris et al., 2018). Furthermore, a recent study found associations between increases in child PFNA concentrations and full scale IQ and perceptual reasoning (Vuong et al., 2019). Altogether, our study and the varied results from relatively few studies indicate no association between prenatal PFAS exposure and child IQ.

4.6. Sex specific effects

Our results suggest effect modification by child sex, where the positive association between component two (PFNA, PFDA and PFUnDA) and verbal working memory were mainly driven by boys. Other studies that are comparable to our study have examined effect modification by child sex, although none of them utilized the same sub-task from Stanford Binet as herein. Most of them report no effect modification (Harris et al., 2018; Høyer et al., 2015, 2018; Liew et al., 2015; Stein et al., 2013; Strøm et al., 2014). Still, one study that examined ADHD symptoms and prenatal PFAS exposure found different results by child sex; some associations were stronger for boys and some were stronger for girls depending on the specific PFAS investigated (Lien et al., 2016). In addition, three studies examining different cognitive or behavioral measures in children in the same cohort, report effect modification by child sex in some of the associations between prenatal and postnatal PFAS exposure and these outcomes (Vuong et al., 2016, 2018, 2019). The mechanistic underpinnings of these observed sex differences is a relatively unexplored area. It could be linked to sex-specific differences in toxicokinetics of PFASs and that PFASs have the potential to disrupt sex hormone homeostasis (Kjeldsen and Bonefeld-Jørgensen, 2013; Mariussen, 2012). A later cognitive development among boys compared to girls could also contribute to the observed difference in boys and girls.

4.7. Dose-response relationships and potential mechanisms

Our findings are in accordance with previous epidemiologic literature showing lack of associations and some inconclusive effects between prenatal PFAS exposure and adverse neurodevelopment. Although the reported associations in our sample are weak and difficult to interpret as clinically meaningful, these associations could be stronger and clearer in other populations where PFAS exposure levels are higher and with larger variability in the outcomes. Reasons for these inconsistencies across studies could be difference in PFAS exposure levels and patterns as well as timing of PFAS measurements during pregnancy. They could also be due to differences in study design and methodology. Another possible reason for the few significant results could be that exposure concentrations are below levels or at the threshold of neurodevelopmental toxicity, as indicated by our findings mainly in the group of highest PFAS exposure compared to the lowest group in the quintile models. This could indicate a dose-response relationship and that our population is in the lower part of this curve, while the top 20% of those with the highest PFAS exposure *in utero* could be at risk of adverse outcomes. Indeed, for some of the associations between the PFASs and nonverbal working memory, there were indications of negative linear dose-response trends in the quintile models, with a monotonic decrease of nonverbal working memory scores as the PFASs increased. Compared with previous studies of prenatal exposure and neurodevelopmental outcomes in children (e.g. Harris et al., 2018; Høyer et al., 2015; Liew et al., 2018b; Oulhote et al., 2016), the concentration levels of PFASs are generally lower in the present study. However, results from a study comparing PFAS levels in several European cohort studies showed that the PFAS levels (PFOA, PFNA, PFUnDA, PFHxS and PFOS) in a sample of pregnant women from the Norwegian MoBa cohort are equal or higher compared to the other cohorts (Haug et al., 2018). The levels reported from the MoBa sample in that study (Haug et al., 2018) are similar to the levels in the present sample.

It appears that effects of PFASs on neurodevelopment found in experimental rodent studies are not easily replicated in human studies. Experimental animal studies have shown that PFASs may be developmentally neurotoxic and endocrine disruptive and that PFAS exposure during critical phases of gestation can affect brain development (Johansson et al., 2008, 2009; Lau et al., 2003; Long et al., 2019; Mariussen, 2012). Mechanistic studies indicate that exposure to PFASs may potentially affect important factors or regulators of brain development such as the thyroid hormone system, calcium homeostasis, protein kinase C, synaptic plasticity, and cellular differentiation (Liew et al., 2018a; Mariussen, 2012). Findings from animal studies show that PFAS exposure may be connected to memory, learning and neuro-motor development and the results indicate that the critical windows of exposure are during early brain development (Mariussen, 2012). However, the exposure levels in animal studies are often higher than in human populations and the contaminants have shorter half-lives in for example rodents compared to humans (Fei and Olsen, 2011; Mariussen, 2012). The higher doses that the animals are exposed to, can cause other detrimental effects like increased mortality and birth defects (Mariussen, 2012). The real-life exposure scenario for the human fetus consists of a range of highly inter-correlated PFASs and other toxicants that can interfere with brain development in combination (Mariussen, 2012; Quaak et al., 2016). Species-specific differences in sensitivity of the various stages of brain development and ability to eliminate compounds in relation to the exposure timing and level, may in part explain these inconsistent findings in experimental versus epidemiological studies. In addition, it could be that only noticeable effects from prenatal PFAS exposure appear when the child is older and their cognitive functions are more developed.

4.8. Study limitations and strengths

Limitations to our study include potential selection bias. The participant rate in the MoBa cohort was 41%, and it was 35% for the clinical assessments of the ADHD Study. The participants in MoBa and the sub studies are in general older, have higher educational level and a healthier lifestyle compared with the general population (Nilsen et al., 2009). This might have led to underrepresentation of children with a higher exposure to risk factors or less variability of the cognitive test scores. Furthermore, since most participants in the ADHD Study were recruited based on high scores on ADHD-related symptoms, it is a

selected group and our study sample thus has more symptoms than a general child population. Another limitation is that we could not account for variation in maternal glomerular filtration rate (GFR), which may be a source of residual bias in our study. GFR influences the urinary excretion of PFASs, which can lead to the appearance of higher PFAS levels among people with lower GFR (Verner et al., 2015). Low GFR during pregnancy has also been associated with lower birthweights (Gibson, 1973; Morken et al., 2014), and lower birthweights with subsequent ADHD symptoms (Lim et al., 2018; Momany et al., 2018). Thus, the potential exists for residual confounding by GFR, which should be addressed in future studies. In addition, interactions between the outcome measures could impact the results, as interactions between ADHD symptoms and estimated IQ with language skills have been reported in another study using data from the ADHD Study (Rohrer-Baumgartner et al., 2014). It should be noted that the estimated nonverbal IQ measure employed in the present study were not significantly related to any of the other included variables (in the expected direction) in the full ADHD Study sample (data not presented). We cannot rule out the possibility that a considerable amount of random error in this variable has cancelled out potential associations between estimated nonverbal IQ and exposure to PFASs. Furthermore, participants with delayed language development were sampled to other sub studies in MoBa, meaning that our language measure is not very discriminative. Hence, regardless of our null findings, this does not prevent detection of associations between PFASs and language related outcomes in other studies.

Our study also has several strengths. Particularly, the use of clinical tests performed by specialized clinicians is a major advantage. In addition, we had a large sample size of 944 mother-child pairs, as well as a nearly equal sex distribution, meaning that we were able to explore potential sex-specific effects, which are lacking in several studies. Further, we investigated PFAS levels as principal components, which allowed us to investigate possible joint influence of correlated PFASs mutually adjusted for the other component. PCA is also a way to reduce the number of tests. To our knowledge, investigating prenatal PFAS exposure and ADHD symptoms with neuropsychological tests among preschoolers has not been done before. We also had the benefit of a large number of relevant covariates collected prospectively during pregnancy, in order to account for residual confounding pathways. Although certain other covariates, such as breastfeeding duration, may influence postnatal exposure and/or neurodevelopmental outcomes through other pathways, since breastfeeding occurs temporally after prenatal exposure, it could not confound prenatal estimates. Other studies have been conducted in MoBa to assess the neurodevelopmental impact of postnatal PFAS exposure (Forns et al., 2015; Lenters et al., 2019), however these studies have not considered prenatal exposure.

5. Conclusion

Based on our results, we did not find consistent evidence to conclude that prenatal exposure to PFASs are associated with ADHD symptoms or cognitive dysfunctions in preschool children aged three and a half years, which is in line with the majority of studies in this area. Our results did however, show some weak negative associations between PFASs and nonverbal working memory, we also observed weak positive relationships with verbal working memory. As exposure to PFASs can be high among small children, more studies measuring both postnatal and prenatal exposure to PFASs with regard to neurodevelopment and cognitive functioning, including measures of working memory, should be performed in future studies. Further studies should also investigate combined effects of the exposed PFAS mixture as well as together with other environmental contaminants. Additionally, there is an imminent need for studies investigating underlying mechanisms linking PFAS exposure to the suspected adverse effects on human brain development.

Declaration of competing interest

None.

Acknowledgements

This research was funded by the Research Council of Norway (MILJØFORSK, project no. 267984/E50 “NeuroTox”), National Institutes of Health (NIH) R01ES021777, and National Institute of Environmental Health Sciences (NIEHS) P30 ES010126. The ADHD Study, from which the present data were drawn, was supported by funds and grants from the Norwegian Ministry of Health, the Norwegian Health Directorate, the South-Eastern Health Region, G&PJ Sorensen Fund for Scientific Research, and from the Norwegian Resource Centre for ADHD, Tourette syndrome and Narcolepsy. The Norwegian Mother, Father and Child Cohort Study is supported by the Norwegian Ministry of Health and Care Services and the Ministry of Education and Research, NIH, and National Institute of Neurological Disorders and Stroke (NINDS) (grant no.1 UO1 NS 047537-01 and grant no.2 UO1 NS 047537-06A1). We are grateful to all the participating families in Norway who take part in this on-going cohort study, and to the staff of the ADHD Study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2019.10.003.

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International Journal of Hygiene and Environmental Health 223 (2020) 238–247

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

The association of water carriage, water supply and sanitation usage with maternal and child health. A combined analysis of 49 Multiple Indicator Cluster Surveys from 41 countries

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ARTICLE INFO

Keywords:
Water
Sanitation
Multi-level modelling
Maternal health
Child health
Mortality

ABSTRACT

Background: Millions of people carry water home from off-plot sources each day and lack improved sanitation. Research on the health outcomes associated with water fetching is limited, and with usage of improved sanitation is inconclusive.

Objectives: To analyse the association of water fetching, unimproved water supplies, and usage of improved sanitation facilities with indicators of women's and children's health.

Methods: 49 Multiple Indicator Cluster Surveys from 41 countries were merged, creating a data set of 2,740,855 people from 539,915 households. Multilevel, multivariable analyses were conducted, using logistic regression for binary outcomes, negative binomial regression for count data and ordinary linear regression for linear data. We adjusted for confounding factors and accounted for clustering at survey, cluster and household level.

Results: Compared to households in which no-one collects water, water fetching by any household member is associated with reduced odds of a woman giving birth in a health care facility (OR 0.88 to 0.90). Adults collecting water is associated with increased relative risk of childhood death (RR 1.04 to 1.05), children collecting water is associated with increased odds of diarrheal disease (OR 1.10 to 1.13) and women or girls collecting water is associated with reduced uptake of antenatal care (β-0.04 to −0.06) and increased odds of leaving a child under five alone for one or more hours, one or more days per week (OR 1.07 to 1.16). Unimproved water supply is associated with childhood diarhhoea (OR 1.05), but not child deaths, or growth scores. When the percentage of people using improved sanitation is more than 80% an association with reduced childhood death and stunting was observed, and when more than 60%, usage of improved sanitation was associated with reduction of diarhhoea and acute undernutrition.

Conclusion: Fetching water is associated with poorer maternal and child health outcomes, depending on who collects water. The percentage of people using improved sanitation seems to be more important than type of toilet facility, and must be high to observe an association with reduced child deaths and diarhhoea. Water access on premises, and near universal usage of improved sanitation, is associated with improvements to maternal and child health.

1. Introduction

Target 6.1 of the UN Sustainable Development Goal on clean water and sanitation is to 'achieve universal and equitable access to safe and affordable drinking water for all', and target 6.2 is to 'achieve access to adequate and equitable sanitation and hygiene for all and end open defecation, paying special attention to the needs of women and girls and those in vulnerable situations', by 2030 (UN, 2015). Equitable or fair access implies that different levels of water supply and sanitation services, or usage of different types of water source and toilet facilities, should not or will not disadvantage specific individuals or households.

In 2017, 785 million people still lacked even a basic drinking water service, defined as one requiring less than a 30 min round trip to fetch water from an improved source. Out of the people lacking a basic service, 206 million people spent over 30 min per round trip to collect water from an improved source (defined as a limited drinking water service) and the remainder relied on unimproved (435 million) or surface water sources (144 million), which most often also require more

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https://doi.org/10.1016/j.ijheh.2019.08.007
Received 16 April 2019; Received in revised form 12 July 2019; Accepted 15 August 2019

than 30 min to walk to, collect water and return home (WHO and UNICEF, 2019). In the same year, 2 billion people lacked a basic sanitation service (WHO and UNICEF, 2019). Off-plot access to water, even as part of a basic service, commonly requires a household member to complete multiple water fetching trips per day or week, with time spent walking to the source, queuing and physically carrying home enough water filled containers to meet their own needs and the needs of other household members (Evans et al., 2013; Geere, 2015). It therefore creates an immediate challenge to obtaining equitable access in comparison to households with water piped into their home, or which is accessible in the yard. It may also disadvantage individuals tasked with fetching water, usually the poorest women and children in low income regions (UN, 2016; WHO and UNICEF, 2017a, WHO and UNICEF, 2019). Many of these women and children also contend with a complete lack of, or unimproved sanitation facilities, which may further challenge their ability to maintain their own and their families' hygiene, health, safety and dignity (WHO and UNICEF, 2017b).

Different levels of access to safe water and sanitation may impact upon individuals and households through a variety of mechanisms or disease transmission pathways. However, epidemiological evidence of the health benefits of access to safe water and sanitation remains equivocal, at least in Low and Middle-Income Countries. For example, recent large scale multi-country randomised controlled trials have not reported clear associations between improvements in water or sanitation provision and either childhood diarrhoea or indicators of malnutrition (Clasen et al., 2014; Luby et al., 2018; Null et al., 2018). Even when randomised controlled trials of water and sanitation interventions have reported improved health outcomes, concerns were raised that such impact may be explainable largely by reporting bias as a result of lack of blinding of participants and investigators (Hunter, 2009; Schmidt and Cairncross, 2009). Equivocal or unclear findings may also be due to the confounding or mediating effects of other pathways leading to poor health, which have not been evaluated or adequately studied.

One aspect of water supply provision that has not been adequately studied, and may confound or mediate any benefits from improved water supply and sanitation interventions, is the impact that having to carry water home from off the site, or 'off-plot' water sources, may have on public health. Studies suggest that the work of water fetching may directly affect the health and wellbeing of the water carrier because it is associated with pain, fatigue and emotional distress (JA Geere et al., 2010; JL Geere et al., 2010 ; Geere et al., 2018; Wutich and Ragsdale, 2008). Through time and opportunity costs, water fetching might also indirectly lead to poorer health. For example, it might limit uptake of health services (Geere et al., 2018), or a person's capacity to engage with occupations which would otherwise enhance personal and family wellbeing, such as paid employment, vending or caring for young children (Wrisdale et al., 2017).

Because women and girls in the poorest families are most often tasked with fetching water (Geere and Cortobius, 2017; Graham et al., 2016; Hopewell and Graham, 2014; WHO and UNICEF, 2017a), it is likely that a differential burden from different levels of water access and the work of water fetching will become apparent as poorer maternal and child health outcomes (Geere et al., 2018; Pickering and Davis, 2012; Porter et al., 2012; Wang and Hunter, 2010), which might occur through a variety of pathways. For example, the time and energy taken for water carriage might reduce women's opportunities to also spend time and energy attending antenatal clinics (McCray, 2004), and antenatal clinic attendance has been shown to be associated with a woman giving birth in a health care facility (Seraphin et al., 2015). Women who lack social support for household water collection may not feel able to spend time away from home to give birth and recover in a health care facility, particularly if they have very young children to care for (Ono et al., 2013). Improved water supply and sanitation within the home might enable a woman to ask for and receive social support in the perinatal period (Subbaraman et al., 2015), which could then facilitate her access to antenatal care, or to travel to and give birth in a health care facility. Alternatively, communities where people have to fetch their own water may not have heath care facilities, or those that do exist may also lack adequate water supply and sanitation services, which could dissuade women from using them (Bouzid et al., 2018). Furthermore, the energy expenditure required for water carriage might exacerbate under-nutrition. During pregnancy or postnatally, insufficient maternal nutrition may impact upon intrauterine growth or breast feeding, to increase risk of child mortality, or children under five having reduced weight for age (WAZ) and height for age (HAZ) z-scores (Black et al., 2008). Unimproved water supply and low levels of improved sanitation usage may also impact on individuals and households through a variety of mechanisms leading to faecal contamination of the environment and within the home, with subsequent transmission of infectious disease (Clasen et al., 2014).

Analysis of existing data to establish whether water carriage, adjusted for unimproved water supply and low levels of use of sanitation, is independently associated with poorer maternal and child health outcomes, is an important step prior to further research into which causal pathways operate in specific contexts. Large scale demographic and health surveys (DHS) and Multiple Indicator Cluster Surveys (MICS) are regularly conducted in many countries and have been used to provide data on access to water and sanitation (Graham et al., 2016; Hopewell and Graham, 2014; Sorenson et al., 2011). However, they have not been used to test hypotheses about associations between water fetching, water supply and sanitation use, and the health and wellbeing of household members. We report an analysis of 49 MICS to test the hypotheses that inadequate access to drinking water and low levels of sanitation use are associated with indicators of poorer maternal and child health.

2. Methods

The primary hypotheses were that adverse maternal and child health outcomes are associated with.

1. Having to carry water
2. Use of unimproved drinking water supplies, and
3. Living in communities with low levels of use of improved sanitation

The key variables linked to the primary hypotheses were age and sex of the person in the household identified as usually responsible for collecting water, whether or not people reported use of an improved water supply, category of toilet/latrine usually used in the house and the proportion of homes in a cluster using improved sanitation.

We analysed data on seven health related indicators or outcome measures. The following health outcomes were tested against all three hypotheses.

1. An increase in the risk of child deaths
2. Higher 2 week prevalence of diarrhoea in children under 5 years of age
3. Decreased WHO weight for age z scores (WAZ)
4. Decreased WHO height for age z scores (HAZ)

In addition, the following indicators of health were tested only for having to carry water (hypothesis 1).

5. Reduced likelihood of giving birth in a health care facility (HCF)
6. Reduced uptake of antenatal care
7. Increased likelihood of a child under 5 being left alone for more than 1 h, for one or more days per week

Data sets from 41 countries derived from 49 MICS conducted between 2009 and 14 and with results reported and publicly available in April 2015, were downloaded after obtaining permission from UNICEF,

Table 1
MICs surveys merged for analysis.

Survey Country (region)	Year	N (households)	N (Individuals)	% sample
Afghanistan	2011	13116	101671	3.7
Argentina	2012	23791	89799	3.3
Barbados	2012	2872	8148	.3
Belarus	2012	8284	23650	.9
Belize	2011	4424	17538	.6
Bhutan	2010	14676	68351	2.5
Bosnia and Herzegovina (Roma Settlements)	2012	1544	5864	.2
Bosnia and Herzegovina	2012	5778	20248	.7
Central African Republic	2010	11756	54281	2.0
Chad	2010	16386	88564	3.2
Congo DR	2010	11393	61543	2.2
Costa Rica	2011	5561	21322	.8
Cuba	2011	9183	35454	1.3
Ghana (Accra)	2010–11	1409	4878	.2
Ghana	2011	11925	54228	2.0
Indonesia (Selected Districts of Papua)	2011	2866	12112	.4
Indonesia (Selected Districts of West Papua)	2011	2816	11533	.4
Iraq	2011	35701	238327	8.7
Jamaica	2011	5960	19277	.7
Kazakhstan	2010–11	15800	54316	2.0
Kenya (Mombasa Informal Settlements)	2009	1016	3216	.1
Kenya (Nyanza Province)	2011	6828	30763	1.1
Lao PDR	2012	18843	98440	3.6
Lebanon (Palestinians)	2011	4747	20983	.8
Madagascar (South)	2012	2968	15556	.6
Mauritania	2011	10116	59993	2.2
Moldova	2012	11354	28852	1.1
Mongolia (Khuvsgul Aimag)	2012	1982	6975	.3
Mongolia	2010	10092	35747	1.3
Montenegro	2013	4052	14691	.5
Nepal (Mid and Far Western Regions)	2010	5899	31753	1.2
Nigeria	2011	29077	150810	5.5
Pakistan (Baluchistan)	2010	11612	88427	3.2
Pakistan (Punjab)	2011	95238	599617	21.9
Saint Lucia	2012	1718	4922	.2
Serbia (Roma Settlements)	2014	1743	9014	.3
Serbia	2014	6191	22194	.8
Sierra Leone	2010	11394	66571	2.4
Somalia (North East Zone)	2011	4777	28604	1.0
Somalia (Somaliland)	2011	4808	30777	1.1
South Sudan	2010	9369	55973	2.0
Sudan	2010	14778	83510	3.0
Suriname	2010	7407	28783	1.1
Swaziland	2010	4834	19843	.7
Togo	2010	6039	30948	1.1
Tunisia	2012	9171	38861	1.4
Ukraine	2012	11321	33761	1.2
Vietnam	2011	11614	44831	1.6
Zimbabwe	2014	15686	65336	2.4
Total		539915	2740855	100.0

using the Statistical Package for the Social Sciences (SPSSv22) software. Separate files recording household level variables related to water access, women's health and child health for each survey were merged by creating unique identity numbers for each case in the spreadsheet, derived from survey, cluster, household and individual line numbers. All surveys were then merged producing a total of 2,740,855 people from 539,915 households included in the final data set (Table 1). All dependent (Appendix A) and independent (Appendix B) variables relevant to this study were checked to ensure that value labels were consistent and transformed if necessary prior to merging surveys and in preparation for analysis.

Health indicators or outcomes included in each survey differed and not all households had members who were relevant cases for each indicator, for example only women of child bearing age were asked about birth history, and only those reporting a live birth can provide data on child deaths. Cases with implausible values or missing data for the dependent or any of the independent variables were omitted from the analyses. The independent variable 'times received antenatal care' was highly skewed and so we used a square root transformation. Several new variables were created by combining or transforming the original MICS variables (Appendix A, Table A7).

SPSS data files were uploaded to MLwiN (v3.01) software (Charlton et al., 2017) to conduct multilevel, multivariable regression analyses of the associations between the key independent variables and maternal and child health outcomes. Where the dependent variable was binary we used logistic regression, where count data we used negative binomial regression and where linear we used ordinary linear regression (Appendix B, table B.8). We conducted four-level analyses in which individual survey respondents (level 1) were nested in households (level 2), which were nested in 'clusters' (level 3: a number of households randomly selected from within an enumeration area, or segment of an enumeration area of the survey), which were nested in surveys (level 4: country and/or surveyed region within a country). Our research aim was to determine the effect of the four key household level variables on health outcomes, as described above. Maternal and child health varies between countries, geographic areas or 'clusters' within

countries and households (Black et al., 2008; Dangour et al., 2013; Goudet et al., 2015). It is therefore likely that contextual factors existing at these levels, but not represented by questions included in MIC surveys and therefore variables in the data set, could be associated with the health outcomes of interest. It is also likely that within clusters, respondents are more similar than people from different clusters, due to shared characteristics and contextual factors. Therefore, the four-level analyses allowed for random effects due to unmeasured contextual factors associated with the clusters in which an individual was situated (at household, enumeration cluster and survey level), and correlations within clusters (individuals within clusters are likely to be more similar than those from different clusters), and adjusted for the effects of individual and household level variables included as covariates in the models (factors known or hypothesised to be associated with the outcomes). To check the robustness of the models we ran fixed effects models for each outcome with country as an explanatory variable. We obtained similar results, but with the random effects models having slightly more conservative parameter estimates and a smaller deviance value indicating a better fit of the models (Appendix C). The analyses enabled us to provide an estimate of the independent association of four key modifiable household level variables with the maternal and child health outcomes of interest in this study.

3. Results

Table 1 (and Appendix A, Table A1-6) list the 49 surveys included in this analysis. The results of the seven regression analyses are shown in Tables 2 and 3. Table 2 shows the results of the regression analyses for child mortality, diarrhoea, and WHO WAZ and HAZ scores. Table 3 shows the results of regression analyses for likelihood of giving birth in a health care facility, uptake of antenatal care, and likelihood of leaving a child under five years of age alone for one or more hours, one or more days per week.

Relative risk of child death was greater in households that fetched water (Table 2). In households where women carried the water the relative risk of child death was 1.05 (95% confidence intervals 1.02–1.08). Where men carried the water, the risk was similar (1.04, 95%CI 1.00–1.07). Where children primarily collected water, there was no increased risk of death. Using an unimproved drinking water source was not independently associated with increased risk of child death. Living in a household where members did not usually use a flush toilet was associated with 9–12% greater relative risk of child death than living in a household where members usually used flush toilets. However, there was little obvious difference in mortality rates between those households using non-flush improved sanitation, unimproved sanitation or practicing open defecation. As the percentage of households in a cluster using improved sanitation increased in communities, the association with child deaths declined. Those children born into communities with > 90% improved sanitation usage were 12% less likely to die than those born into communities with ≤20% usage (Fig. 1).

An increase in the odds of a child under five years of age being reported to have had diarrhoea in the previous two weeks (10–13%) was associated with children collecting water, but not with adults collecting water, when compared to households in which no one collects water (Table 2). Using unimproved drinking water supply compared to improved drinking water supply was associated with an increase in the odds of diarrhoea by 5%. Use of an improved or unimproved toilet and open defecation in comparison to a flush toilet was also associated with an increase in the odds of diarrhoea, with improved toilets associated with a greater comparative increase (16%) than unimproved toilets (11%) or open defecation (5%). Improved sanitation usage was associated with the odds of childhood diarrhoea reducing by 8%, 13% and 21% in the > 60–80, > 80–90 and > 90% categories of coverage respectively (Fig. 2).

A small decrease in children's WHO WAZ scores, which indicate acute undernutrition, was associated with water carriage performed by women, men or boys when compared to non-water fetching households (Table 2). No association was observed between WAZ scores and use of an improved compared to unimproved water supply. The use of non-flush toilets (improved or unimproved) or open defecation compared to flush toilets, was associated with a decrease in WHO WAZ scores. A gradual increase in WAZ score was associated with each higher level of improved sanitation coverage beyond 60% (Fig. 3).

No association between children's WHO HAZ scores, which indicate childhood stunting, and household water fetching or improved water supply was observed (Table 2). Use of non-flush toilets (improved or unimproved) or open defecation compared to flush toilets was associated with a decrease in HAZ scores, and when more than 80% of people within a cluster used improved sanitation an association with increased HAZ scores was observed (Fig. 4).

Water fetching was associated with reduced odds of a woman giving birth in a health care facility (10–12% reduction), compared to non-water fetching households, with little difference according to the age and gender of the person responsible for collecting water (Table 3). A reduction in uptake of antenatal care was observed in households where a girl or woman usually collected water, however, when men or boys usually collected water, the odds ratio for antenatal care uptake was not significantly different from that of women living in non-water fetching households (Table 3). The odds of a child under five years of age being left alone for an hour or more, on one or more days of the week, was increased in households where a woman or female child was responsible for collecting water, but not in those where a man or boy collects water, when compared to households where no one collects water (Table 3).

4. Discussion

We believe that ours is the first study to utilize data from a large number of MICS, and analyse the relationships between water carriage, use of improved drinking water and sanitation, and maternal and child health. We have been able to control for a range of possible confounding factors and allow for random effects at the household, cluster and survey level. We have found that having to carry water home is independently associated with a range of adverse child and maternal health outcomes. In comparison to households where no one must collect and carry water, adults carrying water is associated with increased risk of child death, children carrying water with increased odds of childhood diarrhoea, and adults or boys carrying water with reduced WHO WAZ scores. Women of water fetching households are less likely to give birth in a health care facility, and women or girls collecting water, is associated with reduced antenatal care up-take and children under five being much more likely to be left alone at home. In addition, we report the largest study to date on the associations between toilet facility usage and percentage of households using improved sanitation within a cluster, with a range of health outcomes. Our findings suggest that health benefits are associated with a high percentage of households within a geographic area using improved sanitation. More than 60% usage is associated with reduced diarrhoea and acute undernutrition, and more than 80% usage is associated with reduction of the more severe outcomes of childhood death and stunting. This evidence supports the view that to be effective, WaSH interventions should aim toward sanitation provision and usage for all, and provision of safe water on premises.

Of note in our study, is that whilst use of unimproved water supply, an indicator of water quality, was not associated with risk of childhood death, the need for an adult to collect water from an off-plot source was independently associated with an increased risk of child death. When adults must fetch water, it is likely that in many households children are left unsupervised for the time it takes to walk to a water source, wait in a queue for water and return. Unsupervised children may be at more risk of death from accidental injury, or simply from reduced parental

Table 2
Risk of childhood death, odds of diarrhoea affecting a child under 5 years of age in the previous 2 weeks, and regression parameters for WHO weight for age and height for age z-scores by socio-economic characteristics, demographic variables, water supply, sanitation type, sanitation usage and water carriage.

Independent Variable	Child death RR (95% CI)	p-value	Diarrhoea OR (95% CI)	p-value	WAZ β (95% CI)	p-value	HAZ β (95% CI)	p-value
Fixed part of model								
Person collecting water								
No one	1.00		1.00		0		0	
Male child (< 15 years)	0.99 (0.94, 1.05)	0.828	1.13 (1.02, 1.25)	0.022	−0.05 (−0.09, −0.01)	0.021	−0.03 (−0.09, 0.02)	0.185
Man (15 + years)	1.04 (1.00, 1.07)	0.051	0.98 (0.92, 1.05)	0.602	−0.03 (−0.05, −0.01)	0.012	−0.02 (−0.05, 0.01)	0.139
Female child (< 15 years)	1.00 (0.95, 1.04)	0.871	1.10 (1.02, 1.20)	0.016	−0.00 (−0.04, 0.03)	0.857	−0.01 (−0.05, 0.03)	0.582
Woman (15 + years)	1.05 (1.02, 1.08)	0.001	1.05 (1.00, 1.10)	0.069	−0.02 (−0.04, −0.00)	0.028	−0.01 (−0.03, 0.01)	0.345
Water supply								
Improved	1.00		1.00		0		0	0.729
Unimproved	1.00 (0.98, 1.03)	0.926	1.05 (1.01, 1.10)	0.014	0.02 (0.00, 0.03)	0.055	0.00 (−0.02, 0.02)	
Toilet facility								
Flush toilet	1.00		1.00		0		0	
Other improved	1.10 (1.07, 1.13)	< 0.001	1.16 (1.10, 1.22)	< 0.001	−0.03 (−0.05, −0.01)	0.003	−0.10 (−0.12, −0.07)	< 0.001
Unimproved	1.12 (1.08, 1.16)	< 0.001	1.11 (1.04, 1.18)	0.002	−0.03 (−0.06, −0.01)	0.021	−0.09 (−0.12, −0.06)	< 0.001
Open defecation	1.09 (1.06, 1.13)	< 0.001	1.05 (0.99, 1.11)	0.147	−0.06 (−0.08, −0.04)	< 0.001	−0.08 (−0.11, −0.05)	< 0.001
Improved sanitation usage [c]								
≤20	1.00		1.00		0		0	
> 20 to 40	1.02 (0.98, 1.06)	0.323	0.96 (0.90, 1.03)	0.281	−0.02 (−0.05, 0.01)	0.186	−0.04 (−0.07, −0.00)	0.032
> 40 to 60	1.01 (0.97, 1.05)	0.776	0.93 (0.86, 1.00)	0.056	−0.01 (−0.04, 0.02)	0.441	−0.02 (−0.05, 0.02)	0.368
> 60 to 80	0.98 (0.93, 1.02)	0.251	0.92 (0.86, 1.00)	0.046	0.04 (0.01, 0.07)	0.007	0.03 (−0.00, 0.07)	0.079
> 80 to 90	0.92 (0.87, 0.97)	0.001	0.87 (0.79, 0.95)	0.002	0.07 (0.03, 0.10)	< 0.001	0.07 (0.03, 0.11)	0.001
> 90	0.88 (0.85, 0.93)	< 0.001	0.79 (0.73, 0.86)	< 0.001	0.08 (0.05, 0.12)	< 0.001	0.07 (0.03, 0.10)	< 0.001
Wealth index								
Poorest	1.00		1.00		0		0	
Second	0.96 (0.94, 0.99)	0.004	0.91 (0.87, 0.95)	< 0.001	0.08 (0.06, 0.10)	< 0.001	0.08 (0.06, 0.10)	< 0.001
Middle	0.89 (0.87, 0.91)	< 0.001	0.82 (0.78, 0.85)	< 0.001	0.16 (0.14, 0.18)	< 0.001	0.16 (0.14, 0.18)	< 0.001
Fourth	0.81 (0.78, 0.84)	< 0.001	0.77 (0.73, 0.81)	< 0.001	0.25 (0.23, 0.27)	< 0.001	0.27 (0.25, 0.30)	< 0.001
Richest	0.66 (0.63, 0.68)	< 0.001	0.62 (0.58, 0.66)	< 0.001	0.44 (0.42, 0.47)	< 0.001	0.49 (0.46, 0.51)	< 0.001
Education of household head								
Primary/none	1.00		1.00		0		0	
Secondary +	0.85 (0.83, 0.86)	< 0.001	0.89 (0.86, 0.92)	< 0.001	0.11 (0.10, 0.12)	< 0.001	0.13 (0.12, 0.15)	< 0.001
Area								
Urban	1.00		1.00		0		0	
Rural	0.99 (0.97, 1.02)	0.663	0.92 (0.88, 0.97)	0.001	0.02 (0.00, 0.04)	0.036	0.01 (−0.01, 0.03)	0.476
Sex of household head								
Male	1.00		1.00		0		0	
Female	0.99 (0.97, 1.01)	0.424	0.99 (0.94, 1.03)	0.495	0.06 (0.04, 0.07)	< 0.001	0.05 (0.04, 0.07)	< 0.001
Sex of child								
Male	n/a		1.00		0		0	
Female	n/a		0.92 (0.90, 0.94)	< 0.001	0.06 (0.05, 0.07)	< 0.001	0.08 (0.07, 0.09)	< 0.001
Age in years [a,b]	1.02 (1.02, 1.02)	< 0.001	0.75 (0.74, 0.76)	< 0.001	−0.08 (−0.08, −0.08)	< 0.001	−0.17 (−0.18, −0.17)	< 0.001
β_0 (S.E.)	−3.08 (0.10)		−1.71 (0.13)		−0.72 (0.09)		−0.72 (0.09)	
Random part of model								
Country level variance (S.E.)	0.34 (0.08)		0.60 (0.14)		0.33 (0.08)		0.25 (0.06)	
Cluster level variance (S.E.)	0.17 (0.01)		0.58 (0.02)		0.10 (0.00)		0.12 (0.00)	
Household level variance (S.E.)	0.28 (0.03)		1.23 (0.03)		0.26 (0.01)		0.27 (0.01)	
Individual level variance (S.E.)	0.78 (0.03)		1.00 (0.00)		0.98 (0.01)		1.47 (0.01)	

Note: Number of women reporting child deaths once individuals with missing data excluded = 299, 084 (86.6% of original MICs data), households = 274 145, clusters = 26519, MIC surveys = 40.
Number of women reporting diarrhoea affecting child under 5 years of age in the previous 2 weeks, once individuals with missing data excluded = 290, 176 (78.8% of original MICs data), households = 190 641, clusters = 27 030, MIC surveys = 43.
Number of WHO WAZ scores once individuals with missing data excluded = 230, 406 (84.8% of original MICs data), households = 154 742, clusters = 24 367, MIC surveys = 36.
Number of WHO HAZ scores once individuals with missing data excluded = 217, 210 (80.2% of original MICs data), households = 148 670, clusters = 24, 262, MIC surveys = 36.
RR, relative risk; OR, odds ratio; β, regression parameter; WHO WAZ, World Health Organisation weight for age z-score; WHO HAZ, World Health Organisation height for age z-score; β_0, Y intercept; S.E. = standard error.
[a] For children dead 'age' = age of mother.
[b] For diarrhoea, HAZ and WAZ 'age' = age of child.
[c] % with improved sanitation within cluster.

care when it is needed, for example during illness or when they are very young. In Ethiopia, Gibson and Mace (2006) found that when women's work of water fetching was substantially reduced because of access to tap stands much closer to home, the monthly risk of child death was 50% lower among children of the women with access to the new taps. They suggested that the increase in child survival was most likely due to increased quantity and improved quality of water available for household use, but also greater opportunities for mothers to care for their young children. If the association observed in our study was due to a larger quantity of water being available in non-water fetching households, it is difficult to explain why adults, but not children collecting water, who would be likely to carry even less water than adults, should be associated with an increase in the child death rate. Whilst the increase in risk is not as large as that associated with being in the higher three wealth quintiles, in countries where the under 5 mortality is high a 5% increase in risk independently associated with a modifiable risk

Table 3
Odds of a woman giving birth in a health care facility, uptake of antenatal care and odds of leaving a child under 5 alone > 1 h on 1 or more days per week by socio-economic characteristics, demographic variables and water carriage.

Independent variable	Birth in a health care facility OR (95% CI)	P value	Times received antenatal care β (95% CI)	P value	Child left alone OR (95% CI)	P value
Fixed part of model						
Person collecting water						
No one collects water	1.00		0		1.00	
Male child (< 15)	0.88 (0.79, 0.99)	0.032	−0.02 (−0.07, 0.02)	0.285	0.99 (0.91, 1.08)	0.878
Adult man (15 + years)	0.90 (0.84, 0.96)	0.001	−0.01 (−0.04, 0.01)	0.29	0.98 (0.93, 1.05)	0.605
Female child (< 15)	0.89 (0.82, 0.98)	0.015	−0.06 (−0.09, −0.03)	< 0.001	1.16 (1.08, 1.25)	< 0.001
Adult woman (15 + years)	0.89 (0.84, 0.93)	< 0.001	−0.04 (−0.05, −0.02)	< 0.001	1.07 (1.02, 1.13)	0.003
Wealth index						
Poorest	1.00		0		1.00	
Second	1.33 (1.27, 1.40)	< 0.001	0.06 (0.05, 0.08)	< 0.001	1.02 (0.97, 1.06)	0.459
Middle	1.76 (1.67, 1.85)	< 0.001	0.12 (0.10, 0.13)	< 0.001	1.02 (0.97, 1.07)	0.496
Fourth	2.34 (2.21, 2.48)	< 0.001	0.15 (0.14, 0.17)	< 0.001	0.99 (0.93, 1.04)	0.58
Richest	3.74 (3.47, 4.03)	< 0.001	0.25 (0.23, 0.27)	< 0.001	0.90 (0.85, 0.97)	0.003
Education of household head						
Primary/none	1.00		0		1.00	
Secondary+	1.22 (1.18, 1.27)	< 0.001	0.05 (0.04, 0.06)	< 0.001	0.99 (0.95, 1.02)	0.427
Area						
Urban	1.00		0		1.00	
Rural	0.84 (0.80, 0.87)	< 0.001	−0.05 (−0.07, −0.04)	< 0.001	1.08 (1.02, 1.14)	0.01
Sex of household head						
Male	1.00		0		1.00	
Female	1.15 (1.10, 1.21)	< 0.001	0.02 (0.00, 0.03)	0.012	1.02 (0.98, 1.07)	0.298
Age in years[a]	0.99 (0.99, 1.00)	< 0.001	0.001 (0.00, 0.002)	0.004	1.44 (1.42, 1.45)	< 0.001
β_0 (S.E.)	1.61 (0.43)		2.33 (0.08)		−4.12 (0.26)	
Random part of model						
Country level variance (S.E.)	7.22 (1.63)		0.25 (0.06)		2.78 (0.62)	
Cluster level variance (S.E.)	0.26 (0.01)		0.07 (0.00)		0.87 (0.02)	
Household level variance (S.E.)	0.00 (0.00)		0.06 (0.01)		0.30 (0.02)	
Individual level variance (S.E.)	1.00 (0.00)		0.19 (0.01)		1.00 (0.00)	

Note: Number of women reporting place of birth 100, 505 (85.4% of original MICs data), households = 95 890, clusters = 22 784, MIC surveys = 44.
Number of women reporting times received antenatal care 52, 696 (80.0%), households = 50 689, clusters = 14 904, MIC surveys = 40.
Number of women reporting whether a child under 5 years of age is left alone for an hour or more, on 1 or more days per week = 228, 307 (84.9%), households = 154 705, clusters = 21 617, MIC surveys = 43.
OR, odds ratio; β, regression parameter; β_0, Y intercept; S.E., standard error.
[a] For birth in health care facility and uptake of antenatal care, 'age' = age of woman, for child left alone, 'age' = age of child.

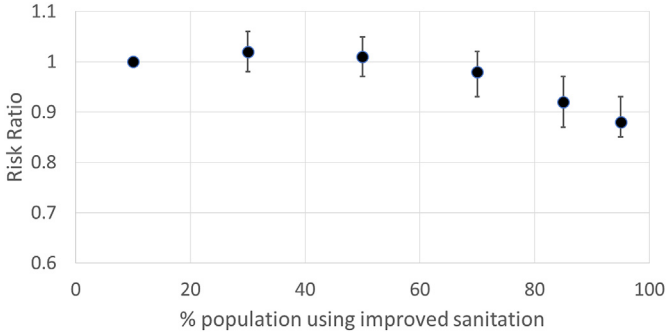

Fig. 1. Relative risk of child mortality by percentage of population using improved sanitation (reference category ≤20% using improved sanitation) Model: negative binomial regression. Covariates: wealth index, education of household head, urban/rural area, sex of household head, age of mother, improved/unimproved water supply, toilet facility, coverage (%) improved sanitation usage, and person collecting water.

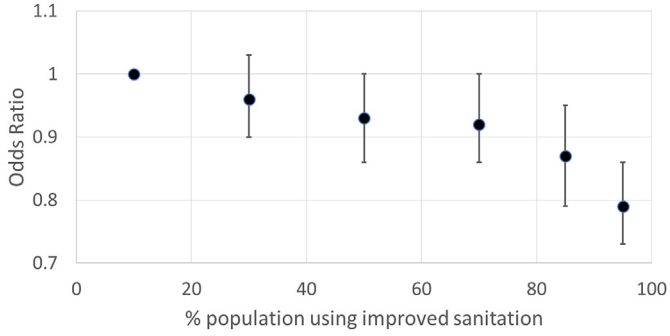

Fig. 2. Odds ratio for childhood diarrhoea by percentage of population using improved sanitation (reference category ≤20% using improved sanitation) Model: logistic regression. Covariates: wealth index, education of household head, urban/rural area, sex of household head, sex of child, age of child, improved/unimproved water supply, toilet facility, coverage (%) improved sanitation usage, and person collecting water.

factor is potentially important. For example our data set includes two surveys from Somalia conducted in 2011, when the under 5 mortality rate for the whole country was reported to be 153.5 deaths/1000 live births or 15.4% (UNICEF, 2019).

Compared to flush toilets, the use of any other type of toilet or open defecation was associated with increased risk of child death. Non-flush toilets of any type had higher relative risk than open defecation, indicating that they may have no benefit or create even greater risk of harm to young children than open defecation. This could occur if toilets are unhygienic, structurally unsafe for a small child to use, or situated in locations which are unsafe for children under five to access (Govender, 2014). Inequitable sanitation access within geographic areas, even where only 20% of households use unimproved sanitation or open defecation, was not significantly associated with a reduction in the risk of child death. This indicates that even a small percentage of households using unimproved sanitation may lead to increased disease transmission through person to person contact or environmental contamination.

The increased odds (10–13%) of children under five having

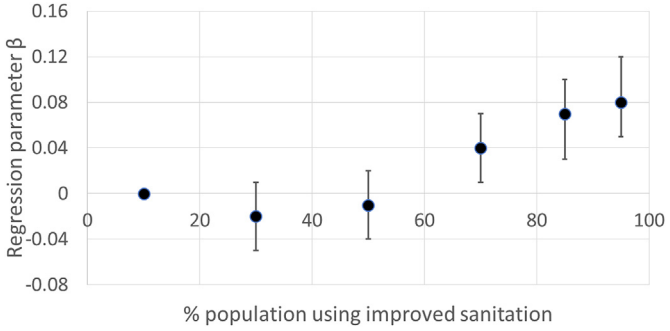

Fig. 3. WHO weight for age z-score by percentage of population using improved sanitation (reference category ≤20% using improved sanitation) Model: linear regression. Covariates: wealth index, education of household head, urban/rural area, sex of household head, sex of child, age of child, improved/unimproved water supply, toilet facility, coverage (%) improved sanitation usage, and person collecting water.

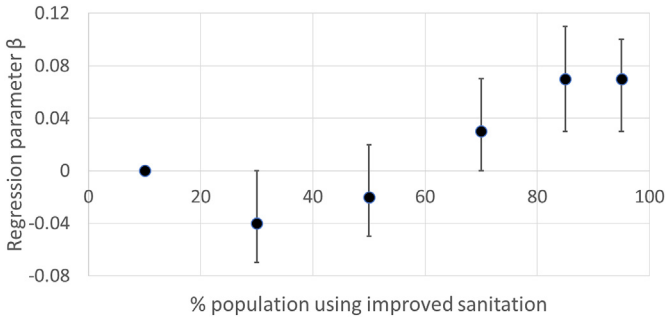

Fig. 4. WHO Height for age z-score by percentage of population using improved sanitation (reference category ≤20% using improved sanitation) Model: linear binomial regression. Covariates: wealth index, education of household head, urban/rural area, sex of household head, sex of child, age of child, improved/unimproved water supply, toilet facility, coverage (%) improved sanitation usage, and person collecting water.

diarrhoea in households where children fetch water compared to households that do not, could simply reflect differing water quality from different source types as reported by Esrey (1996), and that children fetching water away from their home are more likely to be using an unimproved source, and therefore at more risk of diarrheal disease through consumption of contaminated drinking water. However, our analysis adjusted for the 5% increase in diarrhoeal risk from using an unimproved water supply. Furthermore, if use of an unimproved water source were the only reason for the observed association, one would not expect to see significant increases in diarrhoeal disease when children but not when adults collect water, after adjusting for differences in household toilet facilities and sanitation usage. It is known that water quality can deteriorate after collection from a shared source and during storage (Diouf et al., 2014; Jagals et al., 2003) and it's possible that children may be less likely or able to maintain hygienic practices, such as handwashing or cleaning containers adequately prior to refilling them. They may also be more likely to play in or drink untreated water at the source point than adults, and therefore more vulnerable to water borne disease.

Our results showed borderline significance of an association between a woman fetching water and increased risk of diarhhoea (RR 1.05, p = 0.067), whilst men showed no significant association with any increased risk of diarhhoea (0.98, p = 0.602) compared to non-water fetching households. It is possible that by fetching water, adults, and particularly men, may bring larger quantities of water to the house, either because they are simply stronger (Marras et al. 2002, 2003; Stemper et al., 2008) and therefore able to carry more water, or because they are more likely to use equipment or vehicles to collect more water (Geere, 2015). Men are also more likely to collect water when it is located closer to home, and women when it is located further away, such that men may collect larger quantities of water due to proximity of the supply point (WHO and UNICEF, 2017a). A larger quantity of water may enable all household members to improve cleanliness and hygiene practices such as handwashing to reduce the incidence of diarrhoea (Esrey et al., 1989; Hunter et al., 2010). By fetching water, an adult man or woman may also enable other family members, particularly other women but also children, to have more time and energy to engage in household management and chores, including hygiene practices related to washing, cooking and cleaning (Domenech et al., 2012; Rao et al., 2007; Zolnikov and Blodgett Salafia, 2016).

The association of an increased risk of diarrhoea with use of both improved and unimproved toilets, but not with open defecation, when compared to use of flush toilets is surprising. However, 'improved' toilets may not be used by all household members and may not remain functional over time (Clasen et al., 2014), and for these or other reasons may not be effective in preventing faecal contamination of water supplies or the environment (Patil et al., 2014). For example, the difficulties of cleaning, maintaining and emptying 'improved' toilets in which faecal matter is essentially stored near to homes, but not flushed away by water, might mean that it is hard to prevent disease transmission from person to person contact or environmental contamination. Certainly, many latrines, even improved latrines, are not maintained in a hygienic state with faecal smearing especially around the pit (Nakagiri et al., 2015; Simiyu et al., 2017; Sonego and Mosler, 2014). It is highly likely that such filthy latrines add to the risk of enteric pathogens.

Our findings that more than 60% coverage of households using improved sanitation in associated with a significant reduction of childhood diarrhoeal disease, might explain the lack of effectiveness of sanitation programmes reported in the literature. For example Clasen et al. (2014) found that a rural sanitation programme in India, which resulted in a mean 63% of households in the intervention villages having a latrine, had only 11 of 50 intervention villages with ≥50% functional latrine coverage at follow up. The programme was not effective in reducing exposure to faecal contamination or childhood diarrhoea and the authors felt that insufficient coverage and use of latrines were the most plausible explanations for their findings. Their findings are similar to those reported by others in India where there was no difference in household or source levels of *E. coli* contamination between intervention and control groups, and only 41% improved sanitation coverage was achieved in the intervention group (Patil et al., 2014). In Kenya, Null et al. (2018) also found no effect of interventions including improved sanitation on childhood diarhhoea. Whilst adherence to interventions which included improved sanitation was high in their study (78–82% of households), only 33–37% of the same households safely disposed of children's faeces. However, Luby et al. (2018) found that children receiving sanitation, handwashing, nutrition, and combined interventions (but not drinking water chlorination) had less reported diarrhoea. In their study adherence indicated by a functional latrine was very high (96–97%). Further support for this observation that community improved sanitation coverage and usage is more important than individual toilet ownership comes from a recent meta-regression analysis conducted by the World Health Organization (Wolf et al., 2018). This reported larger reductions in diarrhoea in those studies that achieved very high to 100% coverage. Another recent study from Mali also provides strong evidence for this observation (Harris et al., 2017).

Energy expenditure due to the work of water fetching may be important for nursing mothers, and if it affects breast feeding behaviour, might influence childhood nutrition and therefore children's weight for age (WAZ) or height for age (HAZ) scores (Goudet et al., 2015; Keino et al., 2014). WAZ and HAZ scores indicate acute undernutrition and chronic undernutrition or 'stunting' respectively (Dangour et al., 2013). Despite this potential effect, we found a significant but only small

reduction in mean WAZ score in water fetching households associated with adults or boys collecting water, and did not find any association of water fetching with HAZ scores. In contrast to our findings of little to no effect, Gibson and Mace (2006) found that in an area of rural Ethiopia, children under 5 of women with access to water points which reduced the distance and time to fetch water, had significantly increased risk of being malnourished and stunted compared to children of women fetching water in the same area prior to the installation of labour saving taps. They proposed that reduced energy expenditure on water collection supported an observed increase in birth rate (OR 3.78, $p = 0.009$), which as a consequence, meant that smaller, low birth-weight babies were coming to full term and surviving early childhood. Inconsistent findings between studies such as ours and that reported by Gibson and Mace, might be due to contextual factors mediating the effects of water carriage on maternal health and therefore childhood growth.

Others have reported the energy costs of fetching water as moderate to high (Rao et al., 2007) and highlighted that the energy expenditure required for water fetching may become important in 'food-scarce' environments (Domenech et al., 2012). Several other studies also reported fatigue and tiredness affecting water carriers (JA Geere et al., 2010; JL Geere et al., 2010; Hemson, 2007; Porter et al., 2012; Zolnikov and Blodgett Salafia, 2016), and one study (Evans et al., 2013) reported that people who carried water had significantly less (40 min) 'inactivity' time (defined as sleep, resting or watching television) than those who did not carry water. Therefore, whilst findings from a range of studies indicate that the energy expenditure of water fetching may impact detrimentally on pregnant women and mothers, and that reducing the work of water carriage is likely to benefit them, other factors related to maternal or child nutritional intake (Luby et al., 2018; Stewart et al., 2018) and availability of family planning services (Dangour et al., 2013) may determine whether any impact on perinatal or maternal health leads to further impacts on under five weight for age and stunting. We were not able to include any indicators of food intake, nutritional status, feeding programs, birth rates or illness affecting mothers in the analyses, and therefore cannot exclude other possible confounding factors which may have influenced our results.

Dangour et al. (2013) conducted a meta-analysis including 4627 children and found no evidence of an effect of WaSH interventions on WAZ score (mean difference 0.05; 95% CI -0.01 to 0.12) and a borderline statistically significant but small effect of WaSH interventions on HAZ score (mean difference 0.08; 95% CI 0.00 to 0.16). The recent study by Clasen et al. (2014) focusing on a sanitation intervention in India found evidence for small increases in WAZ scores in villages with coverage of ≥50% and households with functional latrines, but no effect on HAZ scores. Our findings that any type of sanitation other than a flush toilet was associated with reduced WAZ and HAZ scores, together with the association of > 60% improved sanitation usage to achieve increased WAZ sores and > 80% usage to achieve increased HAZ scores, support Clasen et al.,'s (2014) recommendations to aim for full latrine coverage and use, and to end open defecation. However, in studies conducted in Kenya (Null et al., 2018), Bangladesh (Luby et al., 2018) and India (Patil et al., 2014), WaSH interventions alone did not improve child growth, and did not add to the improvements observed with nutrition interventions. In our analysis of observational surveys, the effects of water fetching, water supply and sanitation usage were small in comparison to the effects of wealth, which may enable families to secure enough food to optimize maternal and child nutrition. Overall this suggests that sufficient nutrition is of key importance (Black et al., 2008), which may explain why WaSH interventions alone are insufficient to achieve meaningful improvements in childhood growth.

We found that being from a water fetching household was associated with a reduction in the likelihood of a woman giving birth in a health care facility, but with little difference according to who was responsible for collecting water in the household. Ono et al.'s (2013) findings in Western Kenya indicate that decisions about giving birth at home or in a health care facility are complex, may differ according to which family member provides support with water fetching, and is significantly influenced by other factors in addition to social support. These are similar to our findings that wealth, higher education level of the household head, rural location and sex of the household head had the largest odds ratios associated with place of birth. However, our study provides evidence that as a modifiable risk factor, providing water on premises may independently increase the odds of women giving birth in health care facilities, which may be particularly important for women from lower socio-economic groups living in rural areas.

We found that uptake of antenatal care is likely to be lower for women from water fetching households, when a woman or girl is responsible for collecting water. This supports the findings of McCray (2004) who conducted a cross sectional survey of mothers of a child aged 12–23 months, from 327 randomly selected households in Kwazulu Natal, South Africa. They found that if a woman reported fetching water to be a daily activity affected by making a trip to the clinic, she was twice as likely to utilize prenatal care services at a low level, than an average level. Their conclusion was that making water more easily accessible would facilitate access to health care facilities for antenatal care (McCray, 2004). The added perspective from our research, is that where the location of a water source is not likely to change during a woman's pregnancy, help from her husband or sons to fetch water might enable her to receive antenatal care more times, because there was no decrease in uptake of antenatal care when men or boys collect water, compared to up-take of antenatal care in non-water fetching households. This suggests that by fetching water for household use, men and boys can make an important contribution to their family's health, as increased utilisation of antenatal care has been shown to be associated with better maternal and child health outcomes (Lincetto et al., 2006).

The association of an increased odds that a child under five is left alone for more than 1 h, for one or more days per week when women or girls collect water, highlights the challenges of providing child care and supervision when water is not accessed on premises. Qualitative research has highlighted the 'Hobson's choice' that carers face when they must obtain water from off-plot sources, and then choose to either leave their child alone, or take (often carrying) the child with them along what may be an unsafe route (JA Geere et al., 2010; Schatz and Gilbert, 2014; Wrisdale et al., 2017). The lack of change in the odds that a child is left alone when a man or boy collects water may indicate that the woman in the household is relieved of a task which would require her to leave children alone, and that she utilises the additional time to engage in household tasks that allow her to be with her children. When a woman collects water, it is possible that in some households, there may not be another adult at home and available to supervise children. It is also possible that even when living at home, men will prioritise time for income generating or other activities which take place away from home over child minding, and assume that a woman will manage to combine child minding with water fetching.

4.1. Limitations

MIC surveys are cross-sectional studies, which therefore prevent us from being able to confirm causal relationships between variables. The use of completed MICs questionnaires also limits the extent to which we were able to control for bias or confounding in our analyses. The variable 'person collecting water' is indicated by mutually exclusive response categories for the question 'who usually goes to this source to collect the water for your household?' A response option is not available to indicate that multiple people collect water. Therefore data from households where water carriage is performed by multiple people, for example as work shared by women and children, might introduce bias and have a mediating or confounding effect on the association between the person usually carrying water and the outcomes observed in this study. However, this is likely to reduce the strength of association observed and so our findings may underestimate the association. Time

spent finding a place for open defecation (WSP, 2018) might have been a confounding factor affecting the relationship between water fetching and place of birth, up-take of antenatal care, and leaving a child alone. However, inadequate sanitation has been estimated to have much greater economic impacts through direct health costs such as premature death, diarhhoea and stunting than through time costs (WSP, 2018), and it is likely that fetching water for the household is much more time consuming than finding a place to defecate. Several of the outcome variables rely on self-reported information which may introduce reporting bias, however, outcomes such as number of children who have died are likely to be well remembered by respondents, with little gain to be had from intentional misreporting. Considering these limitations, the associations we observed remain plausible, unlikely to have occurred by chance, are strong in some analyses and consistent with the results of other studies, with some evidence of a 'dose-response' relationship for sanitation coverage (Bonita et al., 2006). Therefore, whilst our study cannot demonstrate causal relationships because the data lacks a clear temporal relationship with exposure preceding outcome, and the possibility of bias and confounding cannot be eliminated, it does contribute to the body of evidence supporting causal relationships between the predictor and outcome variables we analysed (Bonita et al., 2006). Further longitudinal cohort studies are required to allow firmer judgements on causation to be made.

The data set included a large number of studies from different countries, which were not conducted at the same time. However, the studies were all conducted within a five year timespan (2009–2014), and utilizing data from all 49 MICS of 41 countries which were available in April 2015 maximizes the generalizability of our results, and the relevance of our findings to global health. The surveys were not designed to specifically test the hypotheses which we have tested, however MICS and DHS data sets from multiple countries conducted at different times have been used to generate descriptive statistics (Graham et al., 2016; Hopewell and Graham, 2014; Sorenson et al., 2011) and to analyse associations between improved water supplies and sanitation usage and incidence of childhood diarrhoea, height and weight (Esrey, 1996). Utilizing a large set of surveys from different countries may increase the risk of variation in study design across surveys, however MICS are conducted after training enumerators to use standardized data collection tools and methods, and with population sampling which is either nationally representative, or representative of a target group or region within a country (UNICEF, 2017). The variables used for analysis in this study were checked and transformed if necessary to ensure that they had identical response options and value labels before data sets were merged for analyses.

5. Conclusion

Data from 49 surveys in 41 countries indicate that the work of fetching water when it is not located in the home or yard is associated with poorer maternal and child health outcomes. Our study is the first to report associations between maternal and child health and the age and gender of the person responsible for collecting water. Water fetching by any household member is associated with reduced odds of a woman giving birth in a health care facility. Adults collecting water is associated with increased risk of childhood death, children collecting water with increased risk of diarrheal disease and women or girls collecting water, with reduced uptake of antenatal care and increased odds of leaving a child under five alone for an hour or more, one or more days per week. We have found that sanitation usage must reach high levels to be associated with a reduction of childhood death and diarrhoea. Our results demonstrate that water access on premises, and high levels of improved sanitation usage, are associated with improvements in maternal and child health and safety.

Acknowledgements and declaration of interest

This analysis was supported, but not funded by the International Labour Organisation and Stockholm International Water Institute. Declarations of interest: none.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2019.08.007.

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